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AUTHORS' ERRATA AND EMENDATIONS

Page 6, sixteenth line from bottom, insert "to 29.3" after "$\alpha = 22.4$,"
Page 16, footnote 2, table 3, Reference (*10*) should be Reference (*9*).
Page 30, reference (8a), "1938" should be "1937."
Page 159, first paragraph, line 8, "(*Ustilago avenae*) (Pers. Jens.)" should be "(*Ustilago avenae* (Pers.) Jens.)"
Page 162, figure 1, "50-c-F-229-2" should be "50-C-F_4-229-2."
Page 180, table 1, last two columns, delete the word "knobs" in the boxheads.
Page 198, eleventh line from bottom, "treatment $2,^4$" should be "treatment $2,^5$."
Page 205, sixth line from bottom, "six" should be "eight."
Page 228, table 1, last column, first line, "7.27" should be "17.27."
Page 273, sixth line from bottom, "Kojantchikov's lever made contact with the heart by means of a piece" should read "Kojantchikov contacted the heart with a piece."
Page 288, "*p*" should be "*n*."
Page 331, last word on page, "Arachi-" should be "Arachis."
Page 352, table 3, first column, "1933-34" should be "1932-34."
Page 383, line 11, "decompose hydrogen peroxide in oxygen and water" should be "decompose hydrogen peroxide into oxygen and water."
Page 390, first line, "table 1" should be "table 2."
Page 427, ninth line from bottom, a shilling mark (/) should be inserted between "difference" and "S. E."
Page 458, table 2, second column, "58" should be in boldface type.
Page 492, line 18, "per million" should be "per milliliter."
Page 738, line 6, omit "contrary" between the words "the" and "was."
Page 758, first and third paragraphs, "μ" should be "mm" wherever used.
Page 802, table 6, last two columns, the headings should be so arranged that they will read: "C divided by percentage of protein" and "Standard error of C divided by percentage of protein."
Page 832, table 1, last column, sixth figure from bottom, "-2.5" should be "$+2.5$."
Page 834, table 3, the sentence immediately under the heading, "Results given in terms of per million of selenium parts" should read "Results given in terms of parts of selenium per million."
Page 888, table 2, under "Susceptible Beets," column 6, "684.1" should be "648.1."
Page 888, table 3, last line, next to last column, "1.1" should be "1.0."
Page 889, table 4, first line, last column, should read minus instead of plus.
Page 892, table 5, under "Turkish Tobacco," "Mar. 3, 1936" should be "Mar. 23, 1936."
Page 928, table 1, column five, third line, ".20" should be ".21."

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NEMATODES INFESTING RED SPIDERLILIES[1]

By G. STEINER[2]

Principal nematologist, Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In various lots of diseased red spiderlilies (*Lycoris radiata* Herb.),[3] submitted in recent years to the Division of Nematology, several parasitic nematode species have been observed. One lot of red spiderlilies, grown near Hamlet, N. C., and submitted in January 1933 by R. W. Leiby, State entomologist, Raleigh, N. C., harbored the bulb or stem nematode, *Ditylenchus dipsaci* (Kühn, 1858) Filipjev, 1936, in the bulbs and leaves, and an undescribed species of *Rotylenchus* in the roots. Three lots were submitted by N. R. Hunt, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. The first of these, from Castle Hayne, N. C., contained the two nemic species just mentioned. The second, originating from Westminster, S. C., was infested by the bud or leaf nematode, *Aphelenchoides fragariae* (Ritzema Bos, 1891) Christie, 1932. The third, from Peachland, N. C., contained the above-named *D. dipsaci*. A fifth lot, from Lexington, S. C., submitted by G. M. Armstrong, of Clemson Agricultural College, had roots infested by the *Rotylenchus* mentioned above.

DITYLENCHUS DIPSACI AS A PARASITE IN THE BULBS AND LEAVES OF THE RED SPIDERLILY

The former *Anguillulina dipsaci* (Kühn, 1858) Gerv. and v. Ben., 1859, was recently made the type species of a new genus, *Ditylenchus*.[4] The name *Ditylenchus dipsaci* is therefore used here as the new scientific term for the well-known bulb or stem nematode. Of the two occurrences of this form in the red spiderlily (for general appearance of this plant, see fig. 1), the one from near Hamlet, N. C., is of special interest, because in this case the infestation probably originated from infested narcissus bulbs growing next to the red spiderlily. Few cases have been observed of such apparent natural transfer of the bulb or stem nematode from one host to another. The close botanical relationship and the similarity of the bulb structure of the red spiderlily to that of the narcissus may account for such a transfer. The disease symptoms in the red spiderlily bulb, which are shown in figure 2, *A*, very much resemble those produced by the same parasite in narcissus bulbs. Of 40 red spiderlily plants examined from this

[1] Received for publication October 9, 1937; issued March 1938.

[2] The author is indebted to Mrs. Florence Albin, of the Division of Nematology, who assisted in the preparation of the text figures, and to E. Artschwager, of the Division of Sugar Plant Investigations, Bureau of Plant Industry, who aided in the interpretation of the root tissues.

[3] This material had been submitted under the name nerine lily (*Nerine sarniensis* Herb.). B. Y. Morrison and S. F. Blake, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, however, determined the host plant to be the red spiderlily (*Lycoris radiata* Herb.). The nerine lily is therefore wrongly recorded as a host of *Ditylenchus dipsaci* (Kühn) Filipjev (see: STEINER, G., and BUHRER, E. M. NOTES ON NEMIC DISEASES. U. S. Dept. Agr. Plant Disease Reptr. 16: 169. 1932. [Mimeographed]).

[4] FILIPJEV, I. N. ON THE CLASSIFICATION OF THE TYLENCHINAE. Helminthol. Soc. Wash., Proc. 3: 80-82. 1936.



FIGURE 1.—Red spiderlilies from Hamlet, N. C., with roots infested by *Rotylenchus brachyurus* n. sp. × ½.

location, however, only 2 harbored the bulb or stem nematode, whereas the *Rotylenchus* species was present in all of them.

The "brown rings" are not so clear-cut in the red spiderlily bulbs as in the narcissus bulbs, and the infection apparently spreads more in a radial direction. During the growing season the nemas are also found in great numbers in some of the leaves (fig. 2, *B*), but here the reaction of the red spiderlily as host is quite different from that of the narcissus. Observations extending over a period of 3 years did

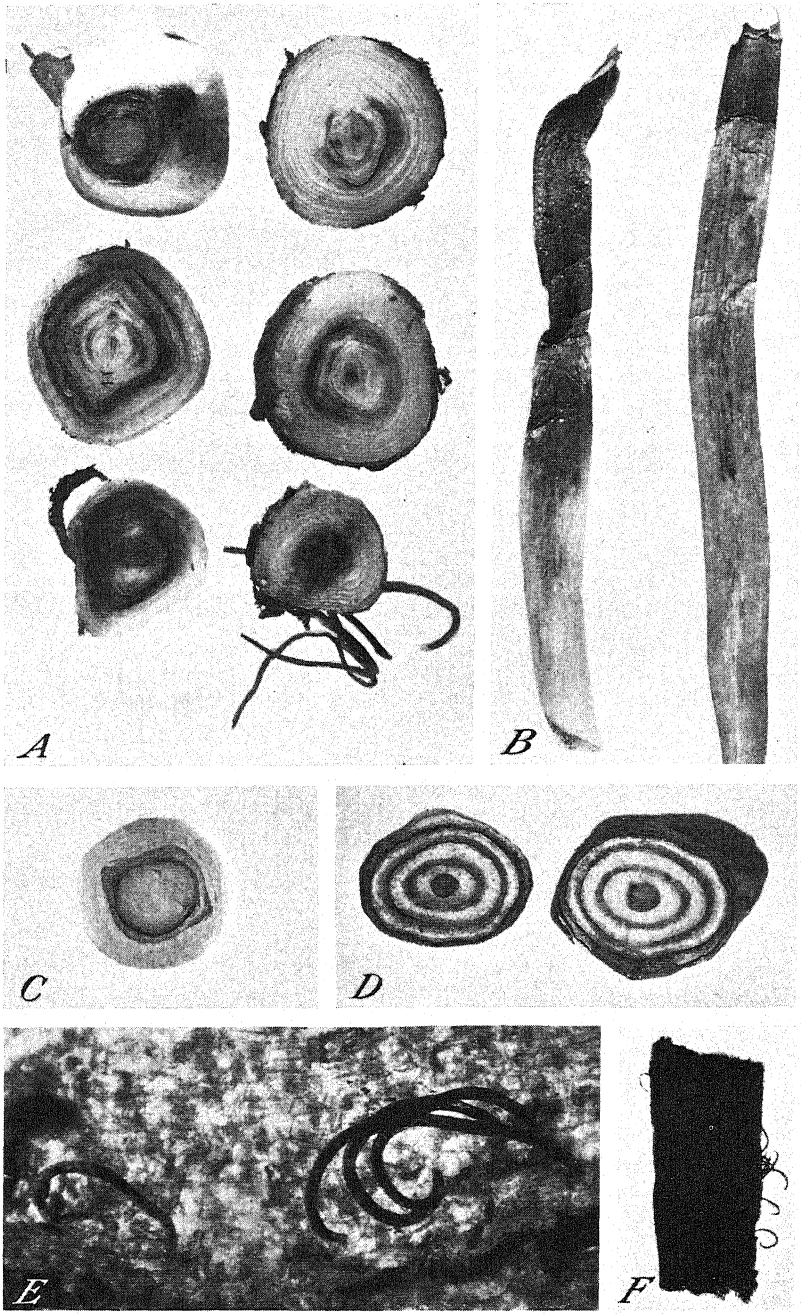


FIGURE 2.—*A*, Red spiderlily bulbs infested with the bulb or stem nematode, *Ditylenchus dipsaci*. Cross sections through two different bulbs; sections through each bulb in vertical arrangement; neck region at top; basal region at bottom. × ⅔. *B*, Red spiderlily leaves infested with the bulb or stem nematode, *Ditylenchus dipsaci*. Actual size. *C*, Cross section through red spiderlily bulb infested with the bud or leaf nematode, *Aphelenchoides fragariae*. × ⅔. *D*, Cross sections through red spiderlily bulb infested with *Cephalobus persegnis*. × ⅔. *E*, Photomicrograph of *Rotylenchus brachyurus* specimens on roots of the red spiderlily. × 65. *F*, Profile view of a root portion of the red spiderlily with *Rotylenchus brachyurus* specimens attached to the surface. × 14.

not disclose "spickles" or otherwise deformed leaves in greenhouse-grown red spiderlilies. The affected areas of an infested leaf turn yellow and then brown, and the leaf finally collapses and decays. As far as observed, this disease is not so virulent in red spiderlilies as it is in narcissus. It develops rather gradually over a period of a year or more but eventually kills the plant. It spreads very slowly to new plants. On the other hand, the nematodes were able to maintain themselves in these hosts under environmental conditions (greenhouse during the hot Washington, D. C., summer) under which narcissus bulbs have been found regularly to lose their parasites. The dimensions of specimens from the red spiderlily bulbs are well within the known range of the species, although close to the lower limit, the size being rather small. The measurements are as follows:

♀: Total length=1.2 to 1.3 mm; α=36. to 51., β=6.7 to 7.8, γ=15.8 to 21., V=80 to 84 percent; distance vulva to anus, about twice length of tail.
♂: Total length=1.1 to 1.3 mm; α=48. to 57.6, β=7. to 8.8, γ=17. to 18.2.

ROTYLENCHUS BRACHYURUS N. SP. AS A ROOT PARASITE OF THE RED SPIDERLILY

There are few published records concerning the plant-pathological significance of the hoplolaims and related forms such as the tylenchorhynchs and the rotylenchs. This group, however, is of much more significance in plant pathology than records would indicate. The writer possesses data showing that representatives of this group are especially numerous in tropical and subtropical regions, where many species affect a variety of crops and trees. The new species of *Rotylenchus* is undoubtedly a parasite of the root (fig. 1); in only one instance were specimens seen attached to the surface of a bulb of the red spiderlily. Unlike the heteroderas, the new species is of the vagrant type of plant-parasitic nematodes, apparently migrating in and out of a root, often feeding from the outside (figs. 2, *F*; 3, *C*). Frequently whole nests of specimens are found, manifestly always in the root exodermis (fig. 3, *A*, *B*), feeding on the cell contents, breaking up the tissue, and opening it for decay.

Specimens have not been observed to penetrate into a root beyond the exodermis. The reasons for this behavior are not known. Suberized cells, which might form a barrier, are apparently absent from the tissue from the exodermis inward. Thickened cell walls delimit the exodermis on its surface (fig. 3, *A*), but they were evidently no hindrance to penetration by the nemas. It may be remarked, however, that in younger roots, with the epidermis still present, these thickened cell walls are seen only at intervals (fig. 3, *B*), the nemas having therefore ample opportunity to penetrate between them. Even so, it is thought that such thickened walls would not form a mechanical obstacle to a species with a buccal stylet of such strength as that of the new species of *Rotylenchus*. The damage caused by these rotylenchs in their hosts is doubtless of less significance than that produced by the heteroderas and similar forms. The rotylenchs attack only the exodermis, and it appears that the host may withstand such injury to a high degree although the production of flowers may be checked to some extent.

Eggs are deposited within the exodermal tissues, apparently in an unsegmented stage, the larvae developing within the plant tissues. That this species leads an exclusively parasitic mode of life is also

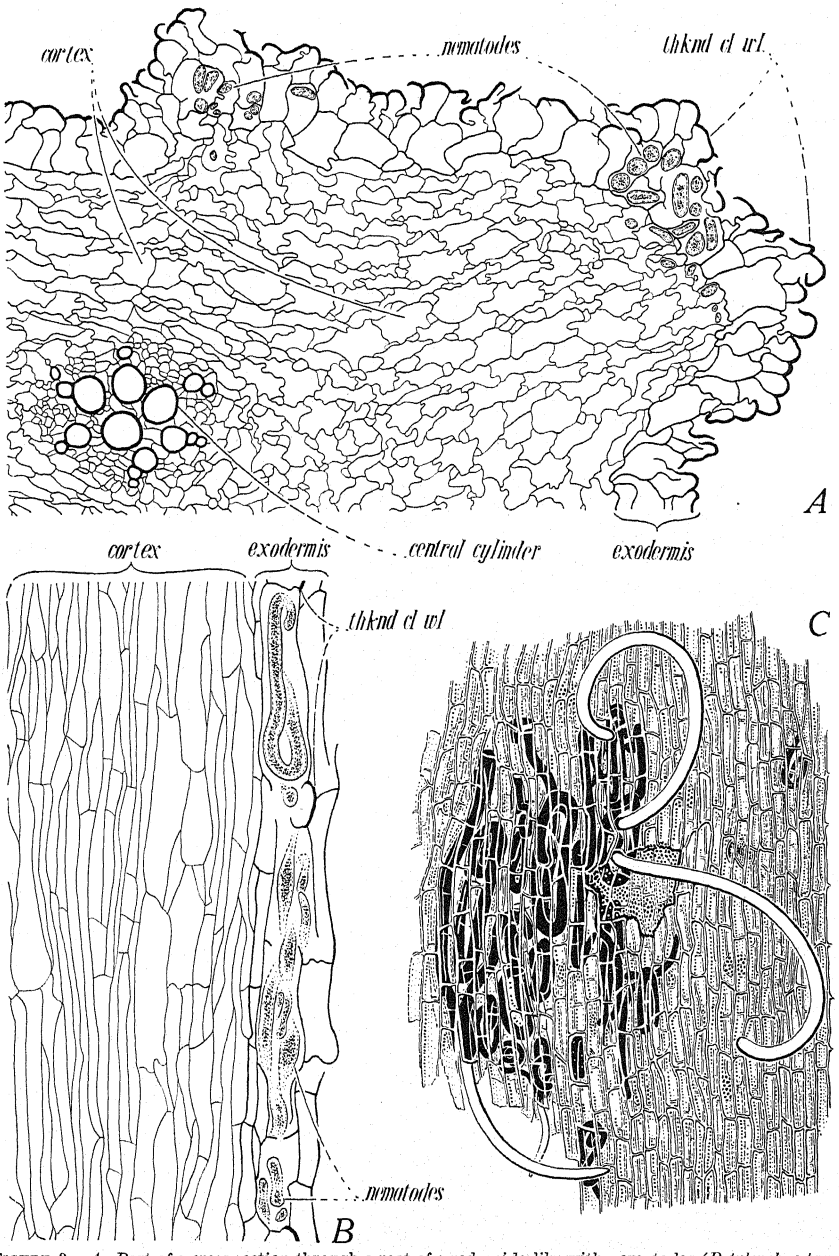


FIGURE 3.—*A*, Part of a cross section through a root of a red spiderlily with nematodes (*Rotylenchus brachyurus* n. sp.) in the exodermis; *thknd cl wl*, thickened walls of cells originally separating the epidermal cell layer (which has disappeared) from the exodermis. × 120. *B*, Longitudinal (tangential) section through part of an infested root of a red spiderlily; nematodes are again found only in the exodermis; *thknd cl wl*, thickened walls of cells, here separated by long intervals. × 120. *C*, Surface view of portion of red spiderlily root with a nest of nematodes (*R. brachyurus* n. sp.), some hanging on the outside with only the head portion inside the tissue. × 75.

suggested by the frailty of the rectum, the apparent reduction of the intestinal cavity, and the opacity of the intestine itself, which is filled with reserve material that makes it appear dark. Although

several hundred specimens were examined, no males were observed, which suggests that the species is either hermaphroditic or parthenogenetic.

In figure 4, *B*, a sketch of the molting head end is given, which demonstrates that here, as in other tylenchs, only the anterior, i. e., the conical portion of the buccal stylet is shed, including the wall of the buccal cavity with its thickened portions to the same distance posteriad. The whole cuticularized framework with its processes is part of the cuticle. The fact that the cylindrical portion and the basal knobs of the stylet are often indistinct in molting specimens, in contrast to the distinctness of the anterior conical part, deserves special mention. These two portions of the stylet are manifestly not only of different origin (ectoderm vs. entoderm) but also of different substance.

TECHNICAL DESCRIPTION

Rotylenchus brachyurus n. sp.

Body usually sickle-shaped or curved in form of loose spiral (figs. 2, *E*; 3, *C*; 4, *K*), cylindrical; tail end broad, even concave (fig. 4, *F*), obtuse; anterior end tapering cephalad of intestine.

Cuticular annulation often rather obscure except on tail end, where distinct. Annuli about 1.5μ wide, laterally interrupted by three longitudinal bands representing the lateral fields. They begin in middle region of unprotruded buccal stylet (fig. 4, *H*), are subdivided by transverse striae in esophageal region (fig. 4, *H*, *I*), then plain (fig. 4, *J*), and end close to tail terminus (fig. 4, *E*, *G*). Annulation on tail following contour of end (fig. 4, *E*, *G*). Scutellumlike phasmid circular to oval, located in latitude of, or slightly posterior to, anus. Lateral bands, anteriorly (fig. 4, *H*) beginning as one, separating into two and then three just in front of middle esophageal bulb, then continuing to tail end (fig. 4, *E*, *G*, *I*, *J*).

Head well set off, conoid obtuse, with five annuli and heavily cuticularized framework (fig. 4, *D*). First annulus behind head, with somewhat heavier cuticularization (fig. 4, *A*, *B*). Buccal stylet strong, about 29μ long, with almost spherical basal knobs, which are only slightly flattened anteriorly; just back of head a distinct conical guiding ring for this stylet (fig. 4, *A*, *B*). Esophagus typical; isthmus and terminal portion very slender, esophageal glands overlapping intestine; end of esophagus set off from intestine, with vestigial, exceedingly small valves (fig. 4, *C*). Intestine opaque, cell walls obscure, cells filled with globular reserve material. Rectum indistinct, almos tobscure; anus only about 10 annuli from tail end.

Excretory pore always in latitude of last esophageal gland, i. e., behind beginning of intestine.

Female sexual apparatus amphidelphic (fig. 4, *K*), anterior as well as posterior branch to left of intestine, each branch about 22 to 25 percent of total body length. Ovaries outstretched, narrow, consisting of few cells in single series. Oviduct very short, uterus with only one egg at a time. Eggs apparently deposited unsegmented. Vagina with thickened walls, leading straight inward. Opacity of intestine often renders close study of female apparatus difficult. Propagation apparently hermaphroditic or parthenogenetic.

Measurements.— ♀: Total length=0.72 to 0.89 mm; α=22.4; β=5.6 to 6.4; γ=47.to 80.; V=59 to 61 percent; eggs=24μ×68μ (fig. 4, *L*).

Diagnosis.—Apparently hermaphroditic or parthenogenetic *Rotylenchus;* cuticle differentiated on lateral fields into three longitudinal bands, two outer ones beginning in region of buccal stylet, separating into three just anterior to middle esophageal bulb, and then continuing past phasmid close to tail end; esophageal portion of these bands areolated by transverse striae; these correlated to annulation of cuticle. Scutellum-shaped phasmid oblong to circular, in latitude of anus. Annulation following contour of tail end. Tail short, broad obtuse, end sometimes concave, its length one-half to three-fourths of anal body diameter. Head with five annuli and strong cuticularized framework. Buccal stylet strong, with spherical, anteriorly slightly flattened but not indented basal knobs. Wall of vestibulum surrounding conical portion of stylet thickened, thus forming a guiding ring.

Type locality.—North Carolina.
Type host.—*Lycoris radiata* Herb.

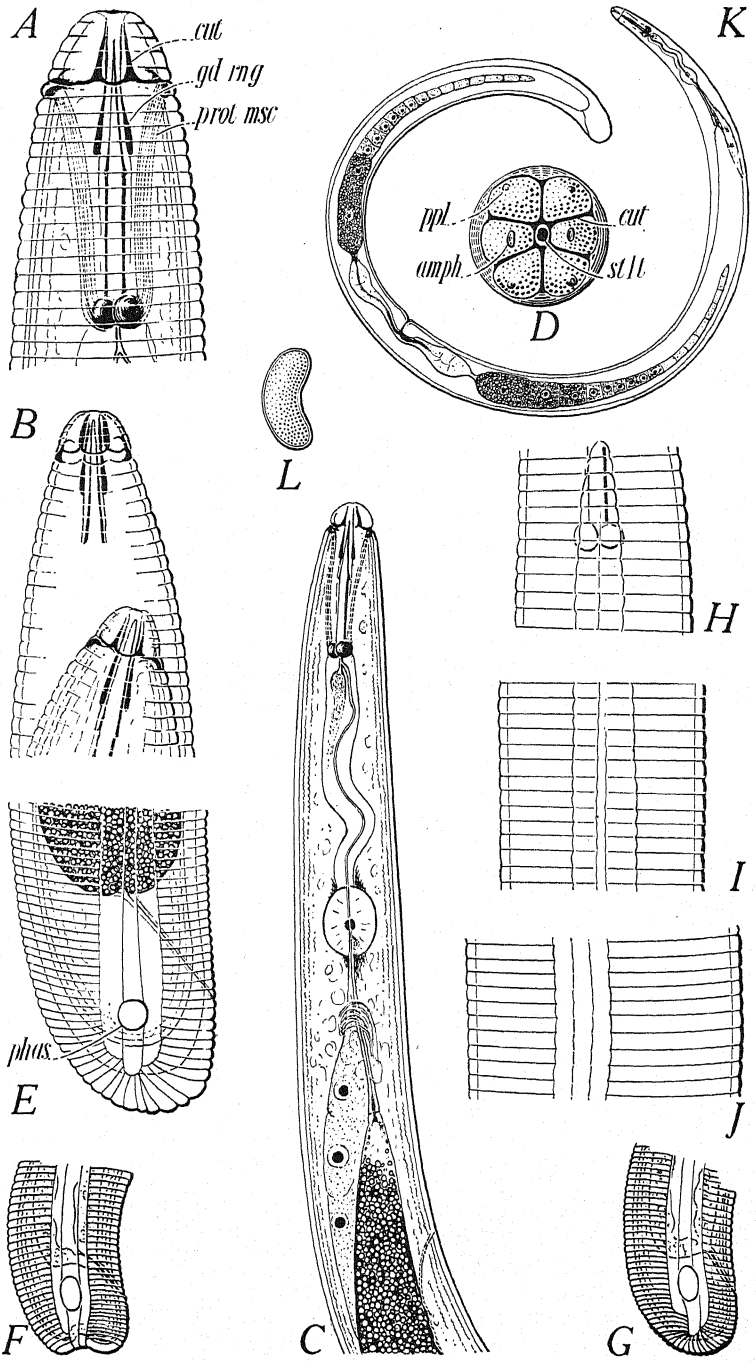


FIGURE 4.—*Rotylenchus brachyurus* n. sp. *A*, Head end: *cut*, cuticular framework; *gd rng*, guiding ring; *prot msc*, protractor muscle of buccal stylet. ×1,750. *B*, Molting head end. ×1,250. *C*, Anterior end of body. ×665. *D*, Front view of head: *amph*, amphid; *ppl*, cephalic papilla; *cut*, cuticular framework; *st/t*, stylet. ×1,710. *E*, Tail end of female: *phas*, phasmidial scutellum. ×1,250. *F*, Tail end of female with slightly concave terminus. ×665. *G*, Tail end of female, another variation. ×665. *H*, Detail of anterior end of lateral bands. ×1,250. *I*, Detail of lateral bands in region of esophageal isthmus. ×1,250. *J*, Detail of lateral bands in postesophageal region. ×1,250. *K*, View of female sexual apparatus in its position in the body. ×200. *L*, Egg. ×200.

APHELENCHOIDES FRAGARIAE AS A PARASITE OF THE RED SPIDERLILY BULB

Figure 2, *C*, represents a cross section through a red spiderlily bulb parasitized by the bud or leaf nematode, *Aphelenchoides fragariae*. The disease symptoms produced in the bulb are also of the "brown ring" type and resemble those produced by *Ditylenchus dipsaci* in red spiderlily bulbs and those so well known in narcissus bulbs. The dimensions of the present specimens agree well with the type; measurements of a female were as follows:

♀: Total length=0.76 mm; $a=51$; $\beta=11$; $\gamma=16.8$; V=67 percent.

CEPHALOBUS PERSEGNIS CAUSING BROWN RING SYMPTOMS ON THE RED SPIDERLILY BULB

In the lot of 40 red spiderlily bulbs referred to above as infested with *Rotylenchus brachyurus*, there was a single bulb which, besides being affected by *R. brachyurus*, on being cross-sectioned exhibited ring symptoms as shown in figure 2, *D*. These proved to harbor numerous specimens of *Cephalobus persegnis* Bastian, 1865.

DORYLAIMUS SUBTILIS AS A ROOT PARASITE OF THE RED SPIDERLILY

Dorylaimus subtilis Thorne and Swanger, 1936, has been previously recorded only from the State of Utah.[5] The finding of rather numerous specimens in roots of the red spiderlily from Hamlet, N. C., suggests, if not a parasitic, at least a semiparasitic, mode of life in plant roots. In male specimens there were six to eight so-called supplements, i. e., series of ventromedian preanal papillae, separated from the adanal papilla by about the length of the spiculum. This species was also observed in roots of the narcissus, variety Olympia, grown in Castle Hayne, N. C.

SUMMARY

Red spiderlilies (*Lycoris radiata* Herb.) in the South Atlantic region of the United States are subject to the attacks of the following plant-parasitic nematodes: *Ditylenchus dipsaci* (Kühn) Filipjev, the bulb or stem nematode; *Aphelenchoides fragariae* (Ritzema Bos) Christie, the bud or leaf nematode; and *Rotylenchus brachyurus* n. sp., a new entoparasite of the vagrant type, attacking the root exodermis exclusively. In one instance, the infestation by the bulb or stem nematode is considered as having originated from narcissus by an apparent natural transfer from that host. The symptoms caused by *D. dipsaci* and *A. fragariae* in the red spiderlily bulb are brownish rings similar to those produced by these parasites in narcissus bulbs. *Cephalobus persegnis* Bastian likewise was found as the apparent cause of brown rings in red spiderlily bulbs, although experimental proof of this relationship is lacking. *Dorylaimus subtilis* Thorne and Swanger was also found as a root parasite but is obviously of little significance as a disease agent.

[5] THORNE, G., and SWANGER, H. H. A MONOGRAPH OF THE NEMATODE GENERA DORYLAIMUS DUJARDIN, APORCELAIMUS N. G., DORYLAIMOIDES N. G. AND PUNGENTUS N. G. Capita Zoologica v. 6, pt. 4, 233 pp., illus. The Hague. 1936.

INITIAL TESTS OF THE DISTANCE OF SPREAD TO AND INTENSITY OF INFECTION ON PINUS MONTICOLA BY CRONARTIUM RIBICOLA FROM RIBES LACUSTRE AND R. VISCOSISSIMUM [1]

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INTRODUCTION

The discovery of white-pine blister rust (*Cronartium ribicola* Fisch.) infections in British Columbia, Canada, in 1921 (*5*) [3] and the extension of the known range as determined in 1922 [4] pointed to the ultimate discovery of the disease in the valuable western white pine (*Pinus monticola* Dougl.) stands of northern Idaho, western Montana, and northeastern Washington, particularly in northern Idaho, adjacent to the international boundary. Preliminary control measures were instituted in the West in 1922.[5] Following the general procedure employed in the East, where blister rust control was already in operation, the Office of Blister Rust Control [6] of the Bureau of Plant Industry confined its initial efforts chiefly to enforcement of plant quarantine regulations, eradication of the highly susceptible European black currant (*Ribes nigrum* L.),[7] and experimental eradication of native forest ribes [8] [9] [10] (*13*). During the same year the Office of Forest Pathology,[11] Bureau of Plant Industry, initiated various studies on the life history and characteristics of the causal organism and the reaction of its hosts.

In the spring of 1928 blister rust was found on western white pine at Newman Lake, Wash., and in the fall of the same year it was noted in Bonner, Idaho, and in Shoshone and Clearwater Counties in northern Idaho, but on ribes only. In 1929 it was found on pines in Clearwater and Shoshone Counties (*13*). These discoveries showed conclusively that blister rust had, as anticipated, invaded the white pine area, and by 1930 local control, as planned in the large-scale blister rust control program (*1*, *3*), was under way.

[1] Received for publication April 2, 1937; issued March 1938.

[2] The authors are indebted to G. B. Posey, formerly of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, who first pointed out the desirability of making these tests, and to J. L. Mielke, of the Division of Forest Pathology, and H. G. Lachmund, formerly of the same Division, for the initiation of this study. W. F. Cummins and A. A. McCready, formerly of the same Division, rendered valuable assistance in the collection of the basic data. Acknowledgments are also extended to Dr. H. T. Güssow, of the Dominion of Canada Department of Botany, and to G. P. Melrose, of the British Columbia Forest Branch, for their friendly cooperation in the work carried out in British Columbia.

[3] Reference is made by number (italic) to Literature Cited, p. 29.

[4] DAVIDSON, A. T. WHITE PINE BLISTER RUST IN BRITISH COLUMBIA. (Report on Canadian conditions and work.) 3rd Western White Pine Blister Rust Conf., Portland, Oreg., 1922, Rept. Proc., pp. 9-22. [Mimeographed.]

[5] POSEY, G. B. REPORT OF WORK PERFORMED BY WESTERN OFFICE OF BLISTER RUST CONTROL. 3rd Western White Pine Blister Rust Conf., Portland, Oreg., 1922, Rept. Proc., pp. 5-9. [Mimeographed.]

[6] Now the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine.

[7] POSEY, G. B. See footnote 5.

[8] The common noun "ribes" is used to include species of both *Ribes* and *Grossularia*.

[9] WYCKOFF, S. N. REPORT OF WORK OF THE WESTERN BRANCH, OFFICE OF BLISTER RUST CONTROL, SEASON OF 1924. Western White Pine Blister Rust Conf., meeting of trustees, Seattle, Wash., 1924, Rept. Proc., pp. 6-8. [Mimeographed.]

[10] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. REPORT OF THE WESTERN BRANCH, OFFICE OF BLISTER RUST CONTROL, TO THE EXECUTIVE COMMITTEE OF THE WESTERN WHITE PINE BLISTER RUST CONFERENCE FOR THE PERIOD APRIL 1 TO NOVEMBER 30, 1923. 20 pp. [Mimeographed.]

[11] Now the Division of Forest Pathology.

Surveys and studies conducted by the Office of Blister Rust Control and by the Office of Forest Pathology indicated that the stream bottoms were the most imminent sources of danger because of the combination therein of concentrations of the most susceptible native ribes with the most favorable climatic conditions for intensification of the rust (*13*). Consequently control effort was directed primarily to the stream types, to which the highly susceptible wild black currant (*Ribes petiolare* Dougl.) and white-stemmed gooseberry (*R. inerme* Rydb.) are confined, and where the less susceptible but numerically important prickly currant (*R. lacustre* (Pers.) Poir.) also occurs (*9, 13*).

The place in the control program of the upland types, characterized by abundant and widely scattered prickly currant and sticky currant (*Ribes viscosissimum* Pursh) (*13*), was not so clear. Tests of these two species had shown them to be relatively low in susceptibility, with the prickly currant apparently the less menacing (*9*). In spite of the low susceptibility of these two species, their wide range in habitat and consequent close association with stands of western white pine made their consideration in the control program imperative. That dense concentrations of these two species should be eradicated was fairly obvious, but where they occurred scattered over large areas the need for their partial or complete eradication was doubtful. In an attempt to secure more definite information on the subject, the Office of Forest Pathology found it possible to begin field work in British Columbia in 1928 on so-called ribes-to-pine spread plots wherein the capacity of the prickly currant and the sticky currant for spreading damaging infection to western white pine would be tested. Similar studies are now being conducted in Idaho, where the results obtained will be directly applicable to the conditions existing in the white pine stands of that State. The present paper reports the results of the investigations that were carried out in British Columbia.

EXPERIMENTAL PLOTS

SELECTION AND PREPARATION

The ideal conditions for the study would have been afforded by a series of areas wherein only one bush or one compact group of bushes of the desired ribes species existed at the center of a group of uninfected pines of uniform distribution. If this central bush should have become infected and spread infection to the surrounding pines, then all resultant pine infection would have been directly traceable to that bush, and if the amount of telia produced were known a measure would have been secured of the capacity of that species for spreading infection under natural conditions. To find a series of such areas was obviously not feasible, leaving as the best alternative the following approximation of the ideal.

Areas of uninfected western white pine of such a size as to permit their ready and thorough examination were located. On each selected area all ribes present within a block 990 feet square (22.5 acres), except those left at the centers to be inoculated, were eradicated as a precaution against extraneous pine infection. Periodic check eradications prevented any reestablishments. As a further precaution all ribes concentrations within a strip approximately 330 feet wide around these areas and, when necessary, within one-half

mile of the area boundaries, were removed. If native ribes did not occur at a desired center, native bushes were transplanted to form the center. These central ribes bushes, of known footage of live stem, were then inoculated in the spring of the year by dusting them with viable aeciospores immediately preceding a rain. Periodically throughout the season the resultant telia production was recorded. On some of the areas two successive tests were conducted (1928 and 1930), 1 year being allowed to elapse between inoculations of the central ribes to permit proper segregation of the resultant cankers on the pines. Plans for further tests on these plots were canceled as soon as it became possible to initiate similar studies in Idaho, and all central ribes were then destroyed.

On each selected area a study plot was established on which all pines within 150 feet (farther for the 1928 infection on two plots) of the central ribes were assigned a number and their locations carefully mapped. Crown length, crown width, and number of needles (*4*) were then determined for each of the trees for the same years in which the central ribes were inoculated. All trees were given several examinations at later dates to locate the cankers which had developed therein as a result of the infection on the central ribes.

While the danger of increasing the spread of the rust in the locality as a result of these experiments was practically nil, such remote danger as may have been involved was eliminated by selecting experimental areas within the infected regions of British Columbia where pine and ribes infection occurred sporadically and where, because of the scattered occurrence of the pine stocking and the high cost of control measures on these rugged sites, control measures were not contemplated.[12] Permission to conduct the experiments in the areas selected was granted by the proper Dominion and Provincial authorities prior to the establishment of the study plots.

CLIMATIC AND ECOLOGICAL CONDITIONS

In the selection of study areas in British Columbia the principal consideration was to locate them so as to approximate the climatic conditions found within the commercial range of western white pine in Idaho. In 1928 seven suitable areas were located, two approximately 40 miles and five approximately 100 miles north of the Idaho line, in the interior white pine belt of British Columbia, the northern extension of the white pine section of northern Idaho, western Montana, and northeastern Washington. The first two areas were near Nelson, at Hall and Apex, respectively, and the last five were near the north end of Slocan Lake at Hunter's Siding, near New Denver. The pertinent climatic data on record for these two localities in British Columbia are compared in table 1 with similar data for stations typical of the range of white pine in Idaho.

[12] DAVIDSON, A. T. RESULTS OF PAST SEASON'S WORK, PRESENT SITUATION, AND FUTURE PLANS. Western White Pine Blister Rust Conf., meeting of trustees, Spokane, Wash., 1924, Rept. Proc., pp. 7–20. [Mimeographed.]

——— BLISTER RUST ACTIVITIES IN BRITISH COLUMBIA. Western White Pine Blister Rust Conf., meeting of trustees, Seattle, Wash., 1924, Rept. Proc., pp. 9–11. [Mimeographed.]

TABLE 1.—*Comparison of general climatic records of stations near experimental plots in British Columbia with records from stations typical of the main commercial white pine section of Idaho*

Month	Precipitation [1]						Relative humidity [1]			Temperature [1]			
	British Columbia		Idaho				British Columbia	Idaho		British Columbia	Idaho		
	New Denver [2] (16 years)	Nelson (29 years)	Priest River (22 years)	St. Maries (27 years)	Avery (18 years)	Musselshell (9 years)	Nelson (11 years)	Priest River (7 years)	Avery (7 years)	Nelson (23 years)	Priest River (22 years)	St. Maries (27 years)	Avery (18 years)
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Percent*	*Percent*	*Percent*	*°F.*	*°F.*	*°F.*	*°F.*
Jan.	3.44	3.14	3.67	3.27	4.19	5.22	88	-------	-------	24	23	28	27
Feb.	2.52	2.11	2.91	2.37	3.19	3.36	85	-------	-------	28	27	32	32
Mar.	2.37	1.82	2.46	2.64	3.71	4.68	74	-------	-------	37	35	39	38
Apr.	1.78	1.53	2.01	1.66	2.48	3.41	64	-------	-------	46	43	47	46
May	2.36	2.23	2.06	2.07	2.29	3.45	61	-------	-------	53	51	54	54
June	2.48	2.61	1.74	1.44	1.90	1.98	67	57	62	60	58	61	60
July	1.08	1.51	.82	.77	.91	1.21	59	46	56	66	64	67	67
Aug.	1.66	1.47	1.17	.82	1.04	.70	64	50	56	64	63	65	66
Sept.	1.80	1.85	1.77	1.27	1.68	1.83	74	63	61	56	53	57	58
Oct.	2.67	2.30	2.42	2.00	2.60	2.75	84	-------	-------	47	43	48	48
Nov.	2.73	2.91	3.72	3.63	3.97	4.47	88	-------	-------	35	33	38	36
Dec.	3.77	3.23	3.94	3.50	3.77	4.64	89	-------	-------	28	25	30	27

[1] Recorded average.
[2] Rain station only. Latest data for New Denver and Nelson, 1930. Latest data for Priest River Experiment Station, St. Maries, and Avery, 1933.

All study areas were located in old burns. The stands were typical of reproduction on burned areas in the white pine region, on which *Salix* spp., *Betula* spp. and *Populus tremuloides* Michx, are the most common deciduous species and western white pine the most abundant conifer. The vegetation had reached that successional stage wherein the conifers were just beginning to overtop and suppress the broadleaf species, except on two of the Hunter's Siding areas. The white pine reproduction on those two areas was much smaller, the burn being more recent and more severe than on the other areas.

The Hall and Apex plots had *Ribes viscosissimum* plants growing naturally at their centers, and three of the plots at Hunter's Siding had *R. lacustre* plants growing naturally at their centers. The two remaining plots at Hunter's Siding were free of ribes. *R. viscosissimum* bushes were transplanted at selected centers on these two latter plots.

The three plots at Hunter's Siding which had ribes growing naturally at the centers will hereafter be referred to as lacustre plot No. 1, lacustre plot No. 2, and lacustre plot No. 3. The two *Ribes viscosissimum* plots near Nelson will be designated as the Hall and Apex plots. The two plots at Hunter's Siding on which ribes plants were set out at the centers will be referred to as viscosissimum plot No. 1 and viscosissimum plot No. 2.

The Hall and Apex plots were natural *Ribes viscosissimum* sites. The soil consisted of well-drained clay loam, with a slight admixture of sand and gravel, and a thin humus layer. Both plots had medium slopes with southerly exposures. Their elevations approximated 3,000 feet. Vegetation was mixed broad-leaved and conifer, chief among the former being *Betula* spp., *Populus tremuloides*, *Alnus* spp., and *Salix* spp., and among the latter *Pinus monticola*, *P. contorta*

Dougl., *Pseudotsuga taxifolia* (Lam.) Britt., and *Larix occidentalis* Nutt. A fairly dense shrub story of *Ceanothus sanguineus* Pursh and *Pachistima myrsinites* Raf. was present on the Apex plot, and scattered bushes occurred on the Hall plot. White pine reproduction within the 150-foot radius on the Hall plot in 1928 ranged from 0.1 to 30.0 feet in height, mainly between 5 and 15 feet, and averaged 7.4 feet, and the stocking was equivalent to 156 pines per acre. Pines within the 150-foot radius on the Apex plot ranged from 0.1 to 34.5 feet high, averaging 7.8 feet, with 217 trees per acre in 1928.

The three *Ribes lacustre* plots at Hunter's Siding varied but little from 2,000 feet in elevation and were situated on gentle slopes with southerly exposures. Here again the sites were typical of the ribes species present. The tree species found were similar to those at Hall and Apex, with the addition of *Populus trichocarpa* Torr. and Gr. and *Acer glabrum* Torr. to the broad-leaved group and *Thuja plicata* D. Don and *Tsuga heterophylla* (Raf.) Sarg. to the coniferous group. The shrub cover consisted principally of *Rubus parviflorus* Nutt., *Pteridium aquilinum pubescens* Underw., *Cornus stolonifera* Michx., and *Pachistima*. The soil was similar on all plots, being a mixture of sand, gravel, and silt covered with a humus layer deeper than that on the Hall and Apex plots. Because of two small, multiforked creeks and numerous springs on lacustre plot No. 1 the amount of moisture on this plot was greater than on either of the two other *Ribes lacustre* plots. However, these streams were not in draws of sufficient depth to appreciably influence local air currents. There was a shallow draw through the center of lacustre plot No. 2 down which water flowed only in the very early spring. A small stream flowed through the center of lacustre plot No. 3 the entire year. This stream meandered through a flat, roughly 100 yards wide, adjacent to a hill arising on the western edge of the plot. In 1928 lacustre plot No. 1 supported 443 western white pines per acre, ranging from 0.3 to 30 feet high and averaging 10.4 feet high; lacustre plot No. 2 had 550 pines per acre, ranging from 0.1 to 43 feet high and averaging 5 feet high; and lacustre plot No. 3 had 164 pines per acre, ranging from 0.1 to 40 feet high and averaging 8.8 feet high.

The two *Ribes viscosissimum* plots at Hunter's Siding were on practically level ground, at an elevation of approximately 1,900 feet. The soil was a well-drained, deep, sandy loam, with a very thin layer of humus. Apparently these areas had been repeatedly burned until practically all organic matter in the top inch of soil had been destroyed. The vegetation was less dense but of the same composition as that found on the Hall and Apex plots. The shrubs *Ceanothus* and *Ribes* were absent. In 1930 the white pines on viscosissimum plot No. 1 ranged from 0.2 to 38 feet high and averaged 3.2 feet high, with 704 trees per acre. In 1930 on viscosissimum plot No. 2 the pines ranged from 0.3 to 35 feet high and averaged 3.9 feet high, with 359 trees per acre.

PROCEDURE

In the spring of 1928 the central ribes on the five plots that had ribes growing naturally at their centers were inoculated in the manner previously employed in the large-scale ribes-susceptibility studies (*9*), and in 1930 the ribes on all seven plots were inoculated. The central plants were periodically examined in the years in which they were not inoculated, as a check on the development of infection upon them.

From the time the plants were inoculated in the spring until their defoliation in the fall, in both 1928 and 1930, ribes examinations were made at approximately 3-week intervals, and the following types of data were recorded for the central plants: Feet of live stem, number of leaves, number of infected leaves, percentage of leaf surface infected on infected leaves, and percentage of infected surface bearing telia. The detailed method of recording ribes infection data has been described by Mielke et al. (*9*). A complete record of infection for each ribes group was thus secured.

Daily records of rainfall and relative humidity were secured from nearby Government weather stations for 1928 and 1930. In 1930 it was possible to have at least one man on the plots at nearly all times; so supplementary weather records were taken daily on each plot from the latter part of June until the ribes were defoliated in the fall. These records included duration of wet pine foliage, wind observations, humidity and temperature readings, and general sky and cloud conditions. Weather conditions undoubtedly exert a great influence on the development and spread of the rust (*10*), and some work has been published on the relation of moisture conditions to pine infection (*12*, *14*). The extreme complexity of this problem, however, makes impossible any accurate analysis of the correlation between pine infection and detailed environmental factors.

Two years after the 1928 ribes inoculations all pines on these plots were given a thorough examination for blister rust cankers. Periodic examinations were made thereafter until the late fall of 1933, when a total of seven examinations had been made. This allowed 3½ years after the 1930 ribes inoculation for all cankers to make an appearance. All cankers found were destroyed before they had produced aecia. The results of these examinations afforded some information on the distance of spread from the central ribes, and also, when correlated with volume of foliage exposed at varying distances, gave the intensity of infection. The following more detailed data were taken on each new canker found: Distance of canker or cankered branch from ground, side of tree, branch or stem, year's growth entered, and stage of development. All branch cankers were further segregated into one of the following categories: Will die before entering the bole; will enter the bole (years); or has entered the bole. Estimates were made on length of time required to kill or damage the tree for all cankers that were formed directly on the bole and for such branch cankers as were eventually to enter the bole, these estimates being based on knowledge of canker behavior and growth rates (*7*). Damage was considered to mean the death or malformation of any part of the tree sufficient to lower its ultimate merchantability. These data provided the necessary information for segregating cankers by year of origin (*6*), affording a measure of the infection and subsequent damage secured from each of the ribes inoculations.

Examination of pines on the plots at both Hunter's Siding and Nelson showed that appreciable infection from the 1928 ribes inoculations had spread beyond the limits of the original 150-foot circular plots. In 1930 the spread was again beyond the 150-foot boundaries of lacustre plots 1 and 3 at Hunter's Siding, but examination of the *Ribes viscosissimum* plots, both at Nelson and at Hunter's Siding, and of lacustre plot No. 2 showed that few if any cankers had originated beyond the 150-foot zone. At Hunter's Siding the areas on which the

R. lacustre plots were located were of such a character that to enlarge the plots would have involved a tremendous amount of labor and was considered impracticable because of the limited available personnel. The character of the areas near Nelson made the enlargement of the plots there a somewhat easier problem. To secure knowledge of maximum distance of spread, the two plots near Nelson, the Apex and Hall plots, were enlarged to a radius of first 200 feet and then 250 feet, as dictated by examination of pines for infection resulting from the 1928 inoculation of ribes. The Hall and Apex plots were not extended beyond the 150-foot radius for examination of pines for infection resulting from the 1930 inoculation.

A very few cankers were found on the areas near Nelson and Hunter's Siding which were clearly not the result of inoculations but of natural spread from associated ribes prior to their eradication from the areas. There is a remote possibility, therefore, that somewhere in the results there may be included a canker or two of doubtful origin, but such cankers are certainly not sufficiently numerous appreciably to alter the results of these studies.

RESULTS

Daily records of precipitation and relative humidity procured from the weather stations nearest to the study areas showed that in both 1928 and 1930 climatic conditions following inoculation of the central ribes were apparently favorable (*2, pp. 209–210; 12, 14*) for the development and uredial intensification of the rust on ribes, especially during the month of June. Conditions were not so favorable for pine infection during the period of telia development and production of sporidia, which normally occurs mainly from July (and even somewhat earlier on *Ribes lacustre*) to early October, precipitation in both years having been below the normal not only of the study localities but also of stations typical of the Washington-Idaho-Montana white pine region. Monthly precipitation records from the Nelson and New Denver weather stations and relative humidity records from Nelson are presented in table 2 as an index to the gross ribes- and pine-infection conditions that prevailed during each infection period.

TABLE 2.—*Comparison of moisture records during periods of possible rust development on ribes for 1928 and 1930, with long-term averages from weather stations nearest experimental plots in British Columbia*

Month	Precipitation [1]						Relative humidity [1]		
	Nelson			New Denver			Nelson		
	1928	1930	29 years	1928	1930	16 years	1928	1930	11 years
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Percent*	*Percent*	*Percent*
April	2.53	1.65	1.53	1.58	1.40	1.78	60	67	64
May	2.50	3.50	2.23	1.69	3.40	2.36	53	65	61
June	4.03	3.56	2.61	4.30	2.56	2.48	68	70	67
July	1.17	.60	1.51	1.75	.78	1.08	65	60	59
August	.49	.68	1.47	.77	1.21	1.66	63	59	64
September	.42	.66	1.85	.25	1.14	1.80	63	68	74
October	3.14	3.15	2.30	4.67	3.54	2.67	79	84	84
November	1.89	1.99	2.91	2.53	.69	2.73	89	90	88

[1] Recorded average.

Table 3 shows the final ribes-infection data recorded for each test plot each year of inoculation, in comparison with the average as determined from previous large-scale ribes-susceptibility tests (*9*). With the exception of the Hall plot for 1930, all plots inoculated showed a higher percentage of leaf surface infected in both 1928 and 1930 than had been previously determined as the average. On all plots inoculated in 1928, the percentages of leaf surface bearing telia in that year were higher than average. In 1930 this figure was again higher than average for all *Ribes lacustre* plots and for the Apex plot, but was decidedly below average on the Hall plot and on the two *R. viscosissimum* plots at Hunter's Siding. The important information derived from table 3, however, is that telia were developed on all plots whenever the central ribes were inoculated, and the percentage of telia produced ranged from relatively high values on the Apex plot and on lacustre plot No. 2 in both years to a relatively low figure for viscosissimum plot No. 2 in 1930.

TABLE 3.—*Percentages of total leaf surface infected and bearing telia on ribes at centers of experimental plots in 1928 and 1930 in comparison with the previously determined average*

Plot	Ribes		Total leaf surface infected			Total leaf surface bearing telia		
	Species	Growth form [1]	1928	1930	Average [2]	1928	1930	Average [2]
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Lacustre No. 1	*R. lacustre*	Part shade	13.2	6.1	4.3	1.8	2.0	0.7
Lacustre No. 2	do	Open	29.4	26.5	3.4	7.5	8.7	.4
Lacustre No. 3	do	Part shade	6.6	8.0	4.3	1.0	5.7	.7
Apex	*R. viscosissimum*	Open	49.9	21.8	6.6	16.4	10.7	3.5
Hall	do	do	11.4	4.2	6.6	4.7	1.7	3.5
Viscosissimum No. 1.	do	do	----	10.2	6.6	----	1.1	3.5
Viscosissimum No. 2.	do	do	----	9.2	6.6	----	.6	3.5

[1] "Part shade"=growing in partial shade; "open"=growing in the open, fully exposed to the sun for most of the day.
[2] Averages from previous large-scale susceptibility tests (*10*).

Data secured on the factors of moisture and ribes infection as they existed on or near the study areas are graphically presented on a daily basis in figures 1 to 6.

The centers of viscosissimum plots Nos. 1 and 2 were but 18 chains apart on an extensive flat. For this reason it was not considered necessary to take separate weather records for each. Figure 6 shows the moisture data on these two plots for 1930, the only year in which their central ribes were inoculated.

In the absence of detailed records, morning dews were arbitrarily assumed (figs. 1–6) to have wet the pine foliage at 4 a. m.

The infections that occurred on the pines of each plot, under the conditions shown in figures 1 to 6, are summarized in table 4, along with the pertinent data on the ribes directly responsible for this pine infection. From this record it is fairly evident that conditions obtaining on all plots (figs. 1–6), with the exception of the Hall plot for 1930, were such as to enable considerable infection to spread from both *Ribes lacustre* and *R. viscosissimum* to the surrounding white pines. Analysis of weather records and ribes-infection data heretofore

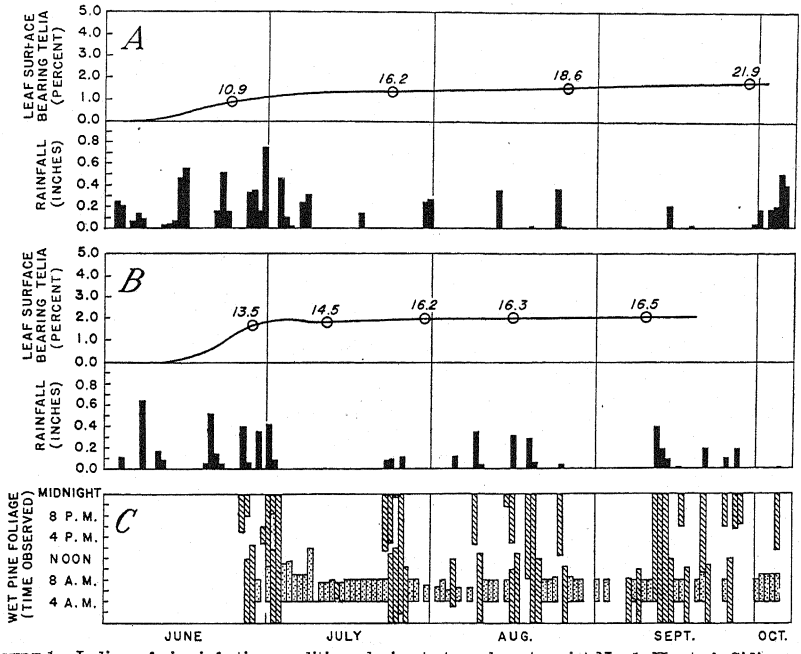


FIGURE 1.—Indices of pine-infection conditions during tests on lacustre plot No. 1, Hunter's Siding, near New Denver, British Columbia. Rainfall and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). Data on telium production were taken only at the times indicated by the points on the curves. The form of curves between points was determined by estimates of the probable development of the rust between these times, based on the stage of development shown, on weather data, and on general knowledge of typical reaction to the rust of the ribes species concerned. The numbers at the points along the curves give the aggregate square inches of leaf surface that had borne telia up to the time indicated. The rainfall data were taken from records of the nearest weather station, New Denver. *C*, Periods of wet pine foliage, as observed on the plot from late June to early October 1930. Diagonal cross hatching represents wetting by rains; stippling, wetting by dews.

presented showed that the pine infection that resulted took place under very ordinary climatic conditions, such as might be expected to occur during almost any summer.

TABLE 4.—*Basis of ribes and pines and infection on each resulting from the 1928 and 1930 inoculations on all study plots*

Plot [1]	Inoculation year	Ribes				Pines				
		Growth form	Bushes	Live stem	Surface bearing telia [2]	Total trees	Average height	Trees infected		Total cankers [3]
			Number	*Feet*	*Square inches*	*Number*	*Feet*	*Number*	*Percent*	*Number*
Lacustre No. 1	1928	Part shade	10	62.4	21.9	719	10.4	79	11.0	127
	1930	do	10	74.2	16.5	715	12.9	52	7.3	101
Lacustre No. 2	1928	Open	5	14.4	29.8	891	5.0	32	3.6	50
	1930	do	[4] 10	24.1	28.4	854	6.5	18	2.1	26
Lacustre No. 3	1928	Part shade	5	328.5	175.0	266	8.8	22	8.3	28
	1930	do	5	431.0	1,658.2	265	10.5	45	17.0	107
Apex (*R. viscosissimum*)	1928 [1]	Open	4	31.0	170.4	658	10.6	54	8.2	283
	1930	do	4	40.0	95.1	334	9.7	5	1.5	5
Hall (*R. viscosissimum*)	1928 [1]	do	5	141.0	181.0	776	8.0	28	3.6	43
	1930	do	5	179.0	69.4	251	9.3	0	0	0
Viscosissimum No. 1	1930	do	20	59.5	24.5	1,141	3.2	21	1.8	25
Viscosissimum No. 2	1930	do	21	80.3	7.3	581	3.9	16	2.8	18

[1] All plots were circular, with a radius of 150 feet, except Hall and Apex, which were extended to 250 feet in radius to include pine infections resulting from the 1928 ribes inoculation.

[2] Calculated by multiplying estimated percentage of leaf surface that bore telia by total leaf area. Total leaf area was calculated from measurements of a portion of the leaves from the central ribes at each test.

[3] The final pine examination was in the fall of 1933, thus allowing ample time for all cankers resulting from both inoculations to make their appearance.

[4] 5 additional bushes were transplanted at the center in 1929 to increase the footage of live stem.

To show variations in pine infection with increasing distance from the central ribes, data for all plots were summarized by concentric circles, each of which had a radius 50 feet greater than the preceding circle. An additional circular division was made at a radius of 117.75 feet to show results on the 1 acre closest to the central ribes. In obtaining these data it was found convenient to map the location of all pines and to show on the same chart the location of all infected trees with their respective number of cankers. For purposes of

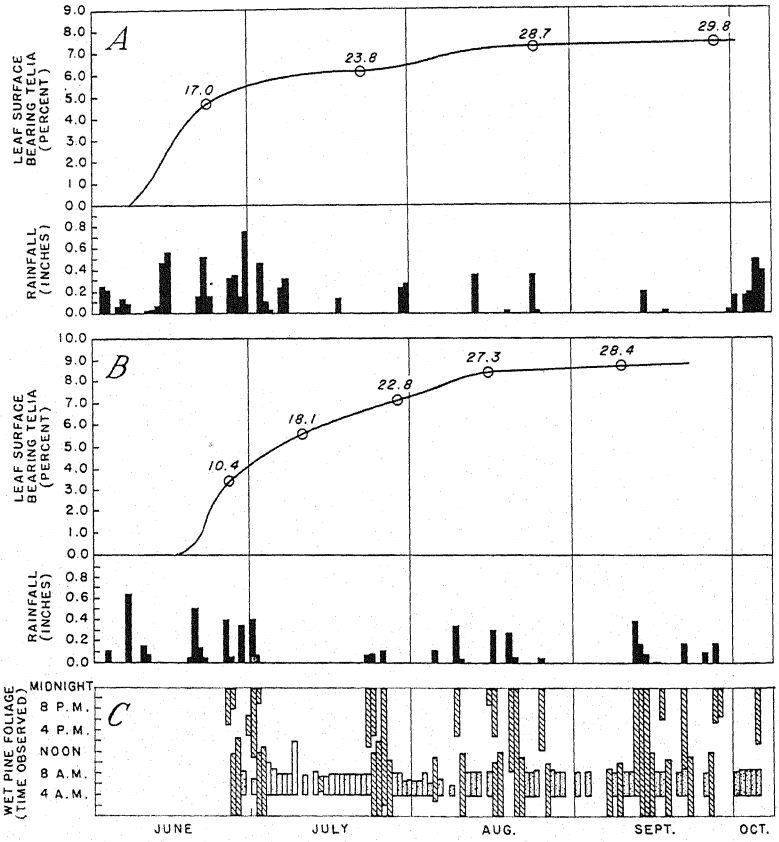


FIGURE 2.—Indices of pine-infection conditions during tests on lacustre plot No. 2, Hunter's Siding, near New Denver, British Columbia. Rainfall and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). *C*, Periods of wet pine foliage as observed on the plot from late June to early October 1930. (For further explanation of details, see legend of fig. 1.)

illustration figures 7 and 8 are given, showing the distribution of cankers resulting from ribes infection on lacustre plot No. 2 for 1930 and on the Apex plot for 1928. Distinctive spread patterns were found in only a few cases, the spread ordinarily assuming comparable proportions in all directions from the central ribes. With lacustre plot No. 2, however, a very definite distribution pattern was obtained in both 1928 and 1930. On this plot the central ribes were located in the bottom of a shallow draw, and a preponderance of cankers was formed along this draw, principally below the ribes, as shown in figure 7. The Hall and Apex plots both had definite slopes and here

again a skewed distribution pattern was secured, there being a strong tendency for the cankers to form on trees down the slope below the source of sporidia, as shown in figure 8 for the Apex plot.

Table 5 summarizes the pine infection and probable resultant damage for all three *Ribes lacustre* plots from both the 1928 and 1930 ribes inoculations. Table 6 similarly summarizes all pine infection and probable damage from the inoculation of *R. viscosissimum* test plots. In table 7 pine infection and probable damage within the circular acre are summarized for each plot individually for each year

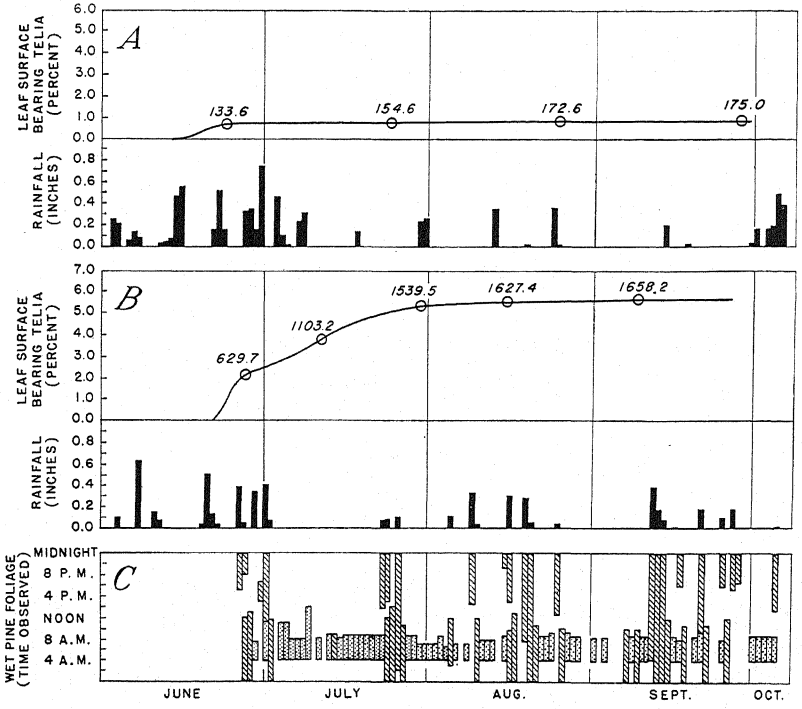


FIGURE 3.—Indices of pine-infection conditions during tests on lacustre plot No. 3, Hunter's Siding, near New Denver, British Columbia. Rainfall and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). *C*, Periods of wet pine foliage as observed on the plot from late June to early October 1930. (For further explanation of details, see legend of fig. 1.)

of ribes inoculation. To complete the picture, the footage of ribes live stem and square inches of leaf surface bearing telia on each plot are included along with the pine-infection data. The individual plots showed wide variation in pine infection, which could not be explained solely upon the basis of probable sporidia production and concurrent moisture conditions. Pine infection is the result of many factors, and to accurately evaluate and correlate all of them would be highly impracticable if not impossible.

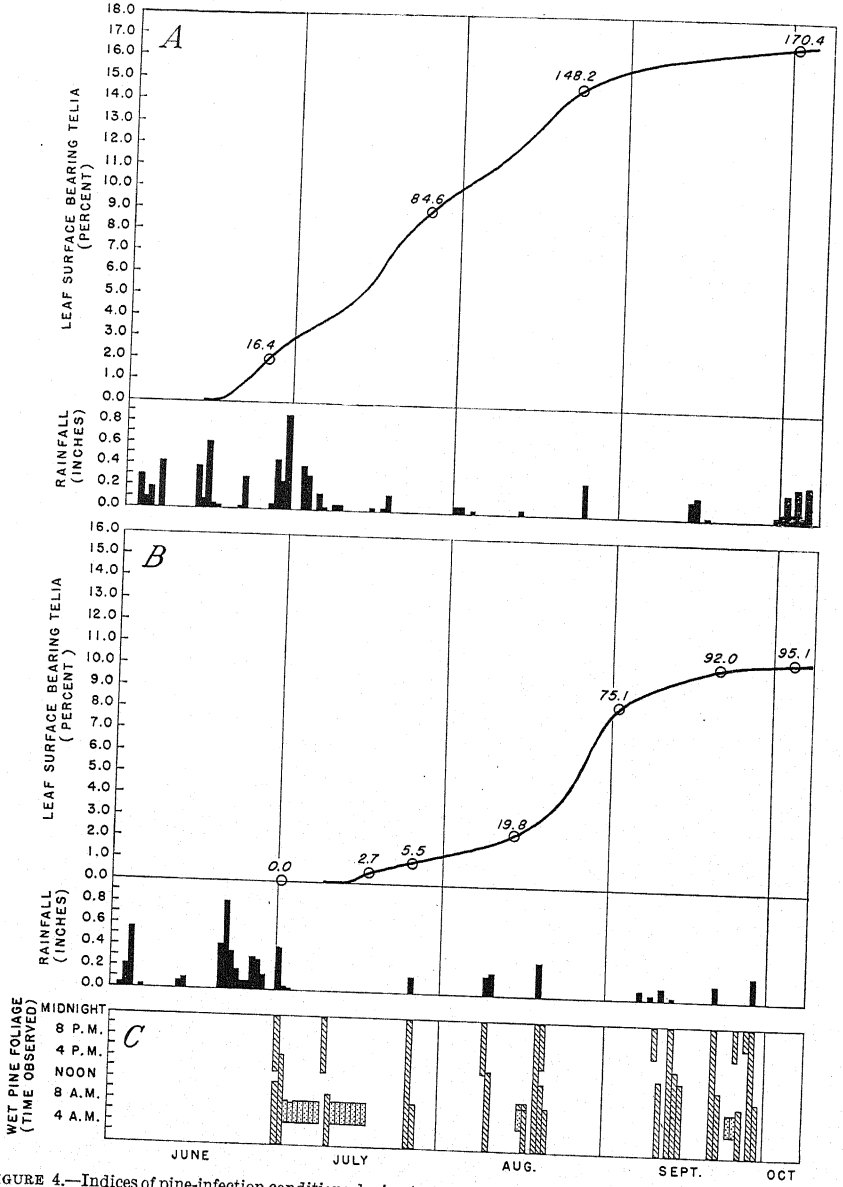


FIGURE 4.—Indices of pine-infection conditions during tests on Apex plot (*Ribes viscosissimum*), near Nelson, British Columbia. Rainfall (records from nearest weather station, Nelson) and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). *C*, Periods of wet pine foliage as observed on the plot from July 1 through September 1930. (For further explanation of details, see legend of fig. 1.)

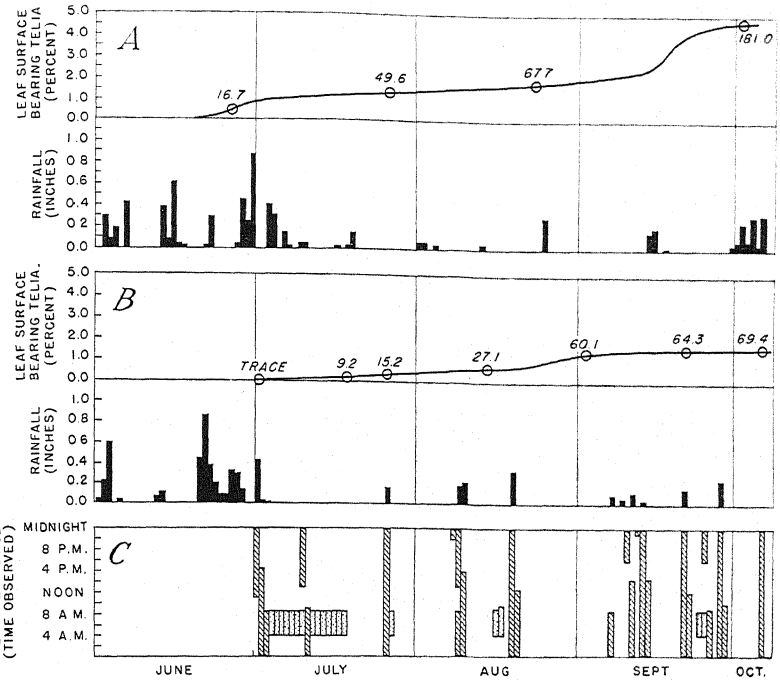


FIGURE 5.—Indices of pine-infection conditions during tests on Hall plot (*Ribes viscosissimum*), near Nelson, British Columbia. Rainfall (records from nearest weather station, Nelson) and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). *C*, Periods of wet pine foliage as observed on the plot from July 1 to early October 1930. (For further explanation of details see legend of fig. 1.)

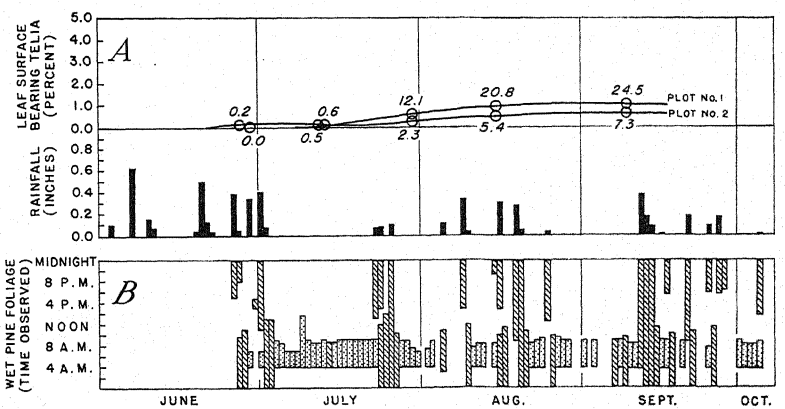


FIGURE 6.—Indices of pine-infection conditions during tests on viscosissimum plots No. 1 and No. 2, Hunter's Siding, near New Denver, British Columbia, 1930. *A*, (upper), Percentage of total leaf surface that was bearing or had borne telia at any particular time; (lower), rainfall data from records of nearest weather station, New Denver; *B*, periods of wet pine foliage as observed on the plots from late June to early October. (For further explanation of details, see legend of fig. 1.)

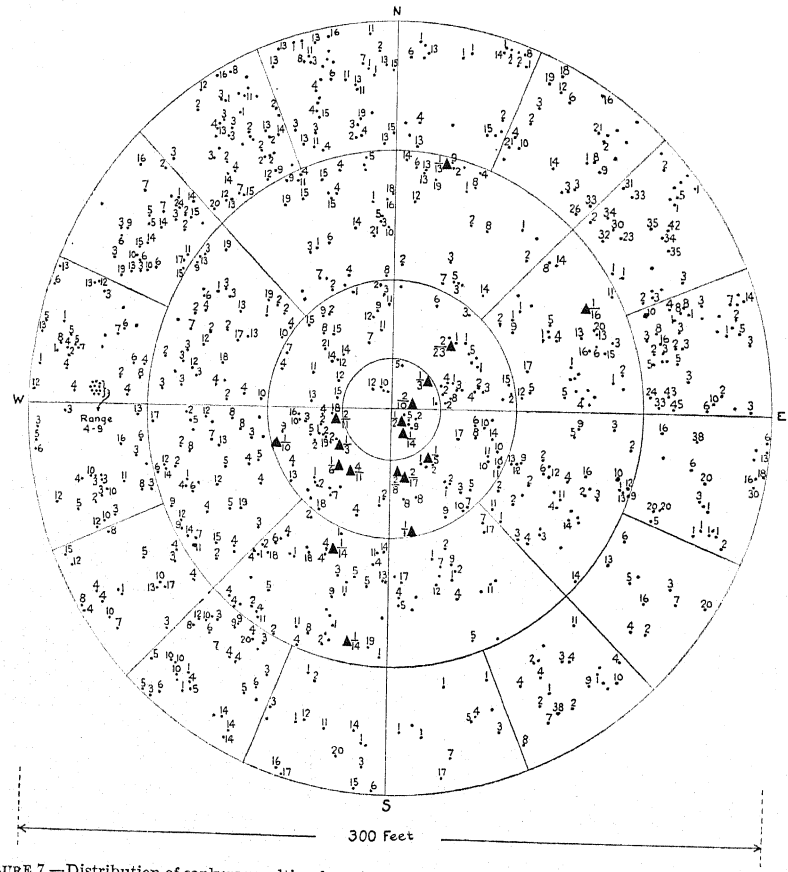


FIGURE 7.—Distribution of cankers resulting from infection in 1930 on *Ribes lacustre*, on lacustre plot No. 2, Hunter's Siding, near New Denver. Dots represent uninfected trees; tree heights of 1 foot or more are shown by numbers immediately above or next to each dot; infected trees are shown by solid triangles, the fractions adjacent to which give number of cankers over tree height.

TABLE 5.—*Spread and intensity of infection and prospective damage* [1] *to pines resulting from sporidia produced on Ribes lacustre, based on 6 tests on 3 plots in 1928 and 1930 at Hunter's Siding, British Columbia*

Distance from central ribes (feet)	Trees							Cankers			
	Total	Average height	Total infected		Total that will be damaged [2]		Infected trees that will be damaged [2]	Total [3]	Damage cankers [4]		Average time required to damage [1]
	Number	*Feet*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Number*	*Percent*	*Years*
0–50	476	7.8	68	14.3	31	6.5	45.6	158	42	26.6	10
50.1–100	1,412	9.1	100	7.1	32	2.3	32.0	139	34	24.5	11
100.1–150	1,821	8.3	80	4.4	36	2.0	45.0	142	42	29.6	12
0–100	1,888	8.8	168	8.9	63	3.3	37.5	297	76	25.6	11
0–117.75 [5]	2,753	8.9	206	7.5	80	2.9	38.8	346	94	27.2	11
0–150	3,709	8.6	248	6.7	99	2.7	39.9	439	118	26.9	11

[1] Damage=killing or injury sufficient to render the trees worthless for timber or timber production.
[2] Trees that would have been damaged if cankers formed thereon had not been cut out.
[3] Includes both damage cankers and those that would have died without causing serious injury.
[4] Cankers which would have caused damage if they had not been cut out.
[5] 1 circular acre.

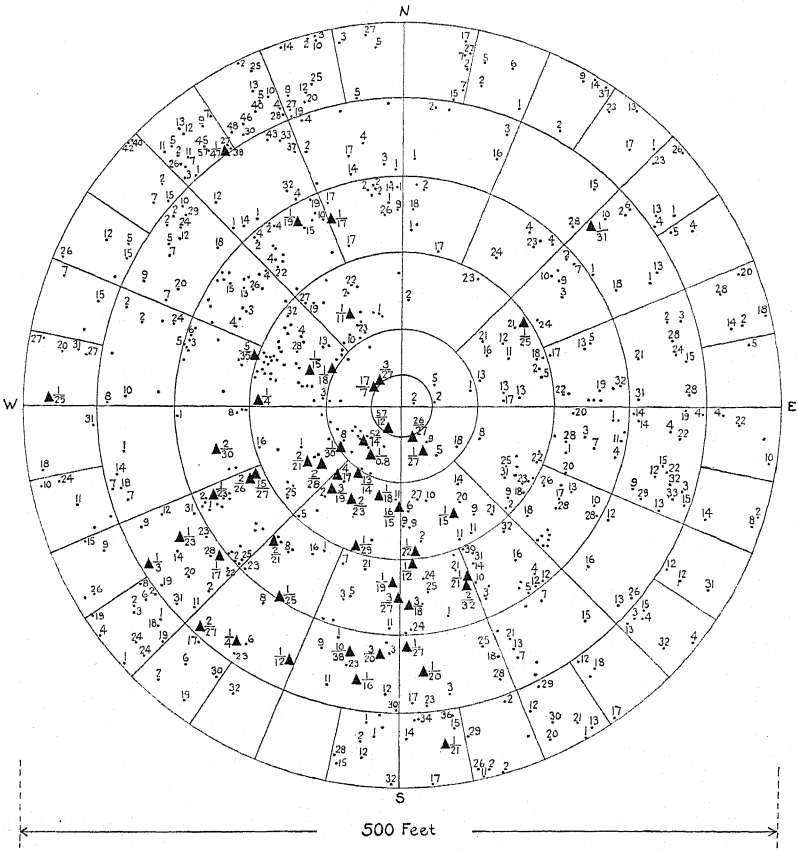


FIGURE 8.—Distribution of cankers resulting from infection in 1928 on *Ribes viscosissimum*, on Apex plot, near Nelson. Dots represent uninfected trees; tree heights of 1 foot or more are shown by numbers immediately above or next to each dot; infected trees are shown by solid triangles, the fractions adjacent to which give number of cankers over tree height.

TABLE 6.—*Spread and intensity of infection and prospective damage* [1] *to pines resulting from sporidia produced on Ribes viscosissimum, based on 4 tests on 2 plots in 1928 and 1930 near Nelson and 2 tests on 2 plots in 1930 at Hunter's Siding, British Columbia*

Distance from central ribes (feet)	Trees							Cankers			
	Total	Average height	Total infected		Total that will be damaged [2]		Infected trees that will be damaged [2]	Total [3]	Damage cankers [4]		Average time required to damage [1]
	Number	*Feet*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Number*	*Percent*	*Years*
0–50	345	4.9	42	12.1	24	6.9	57.1	220	34	15.4	9
50.1–100	1,006	5.8	29	2.8	12	1.1	41.3	64	16	25.0	11
100.1–150	1,560	5.4	32	2.0	9	.6	28.1	57	10	17.5	12
150.1–200 [5]	341	10.1	17	5.0	6	1.8	35.3	29	7	24.1	14
200.1–250 [5]	489	10.6	4	.8	1	.2	25.0	4	1	25.0	21
0–100	1,351	5.5	71	5.2	36	2.6	50.7	284	50	17.6	10
0–117.75 [6]	1,832	5.8	83	4.5	43	2.3	51.8	315	58	18.4	10
0–150	2,911	5.5	103	3.5	45	1.5	43.6	341	60	17.5	10
0–200 [5]	3,252	6.0	120	3.7	51	1.6	42.5	370	67	18.1	11
0–250	3,741	6.6	124	3.3	52	1.4	41.9	374	68	18.2	11

[1] Damage=killing or injury sufficient to render the trees worthless for timber or timber production.
[2] Trees that would have been damaged if cankers formed thereon had not been cut out.
[3] Includes both damage cankers and those that would have died without causing serious injury.
[4] Cankers which would have caused damage if they had not been cut out.
[5] The Hall and Apex plots were extended to a 250-foot radius to trace out the pine infection for 1928. The other four tests included in this table extended 150 feet from the central ribes. [6] 1 circular acre.

TABLE 7.—*Pine infection and subsequent damage within 1 circular acre surrounding a central group of ribes, resulting from a single inoculation of the ribes on each of the 7 test plots*

Plot	Inoculation year	Ribes		Pines on the central circular acre [1]						Total cankers [3]
		Live stem	Leaf surface bearing telia	Total	Average height	Infected		Damaged [2]		
		Feet	*Square inches*	*Number*	*Feet*	*Number*	*Percent*	*Number*	*Percent*	*Number*
Lacustre No. 1	1928	62.4	21.9	557	10.5	64	11.5	32	5.8	109
	1930	74.2	16.5	555	13.4	39	7.0	9	1.6	45
Lacustre No. 2	1928	14.4	29.8	618	5.6	30	4.9	17	2.8	47
	1930	24.1	28.4	567	6.6	18	3.2	5	.9	26
Lacustre No. 3	1928	328.5	175.0	230	8.1	16	7.0	3	1.3	22
	1930	431.0	1,658.2	226	9.8	39	17.3	14	6.2	97
Apex (*R. viscosissimum*)	1928	31.0	170.4	232	8.9	29	12.5	13	5.6	239
	1930	40.0	95.1	194	10.7	3	1.5	2	1.0	3
Hall (*R. viscosissimum*)	1928	141.0	181.0	161	7.7	18	11.2	6	3.7	33
	1930	179.0	69.4	159	9.3	0	.0	0	.0	0
Viscosissimum No. 1	1930	59.5	24.5	707	3.1	18	2.6	16	2.3	23
Viscosissimum No. 2	1930	80.3	7.3	379	4.0	15	4.0	6	1.6	17

[1] 1 circular acre has a radius of 117.75 feet.
[2] Trees that would have been damaged if cankers formed thereon had not been cut out.
[3] Includes both damage cankers and those that would have died without causing serious injury.

The number of cankers formed at increasing distances from the central ribes, as averaged for all tests, is shown by the curve in figure 9, in which number of cankers per million needles is plotted over distance from plot center in feet. A measure of the intensity of pine infection at varying distances from the plot center was also secured by plotting percentage of total cankers within a given zone, divided by percentage of total needles within that same zone, over distance from plot center in feet. These data are graphically presented in figure 10 for each plot for each year of inoculation. These curves show that in all cases the intensity of infection fell to almost negligible values between 50 and 60 feet from the central ribes irrespective of how great the intensity may have been closer to the ribes.

In analyzing these curves it should be borne in mind that, although there is a decided reduction in intensity of infection beyond 50 or 60 feet from the central ribes, there is still a considerable number of cankers formed at even greater distances. Trees close to the central ribes are very likely to receive new damaging infections with each successive year of ribes infection—a duplication of damage that is not particularly significant, because one damage canker alone is sufficient to cause the loss of a tree. Duplication of damage is less likely to occur on the more distant trees, as the sporidia will be more and more widely dispersed with increasing distance from the ribes. For example, table 5 shows that 31 trees were damaged in the 0- to 50-foot circles, 32 were damaged in the 50.1- to 100-foot circles, and 36 were damaged in the 100.1- to 150-foot circles. It is therefore apparent that, while percentage of damage may decrease rapidly beyond the 150-foot circle, the total damage beyond this point may exceed that within 150 feet of the ribes.

DISCUSSION

The artificial inoculation exposed the central ribes on the test plots to a greater volume of aeciospores and resulted in heavier infection and subsequent telia production than would occur in nature unless the ribes were exposed to a nearby and abundant source of aecia. Such

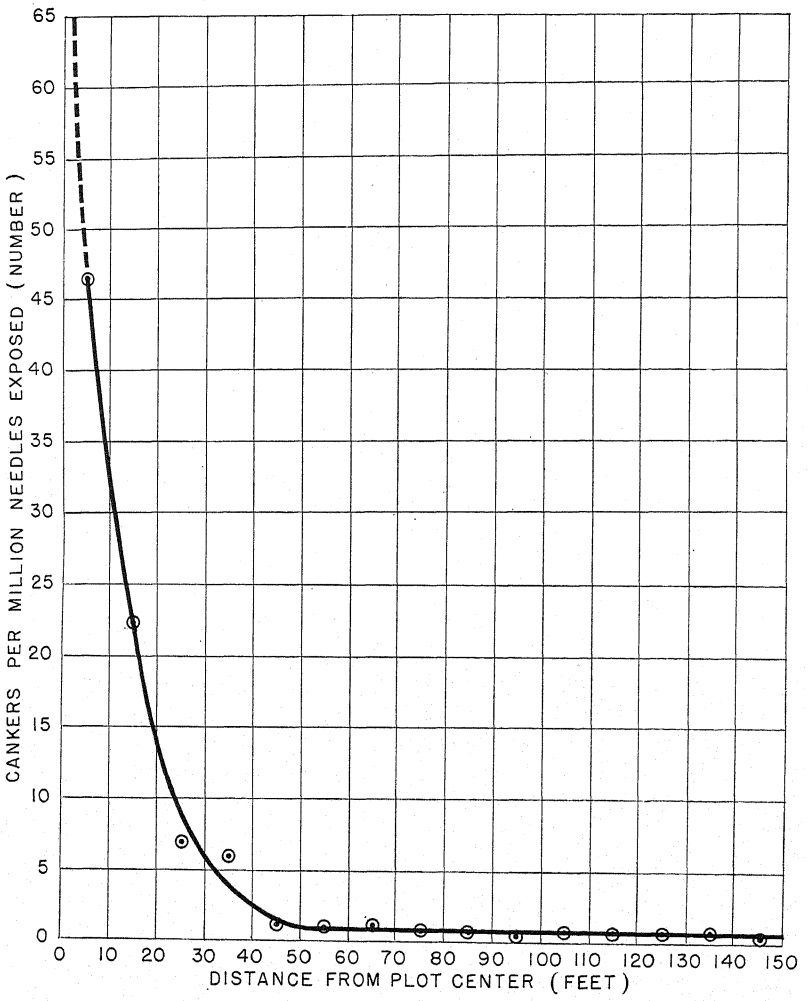


FIGURE 9.—Average number of cankers per million needles exposed in 10-foot zones out from the central ribes, for all plots combined.

association would be only occasional in the first stages of natural invasion of the rust into a previously rust-free area. In older centers of pine infection such associations might readily occur with alarming frequency. The time required for such an aecial supply to be built up in a previously rust-free area is extremely variable and depends to a considerable extent on the original intensity of ribes infection, the ribes-pine association, and the climatic conditions that prevailed

during these developmental years. In view of the great importance of the stage of development of ribes leaves at the time of inoculation to their susceptibility, as shown by Lachmund (*8*), it is possible that natural inoculations, where ribes are growing near fruiting cankers, might in some cases result in greater development of the rust and

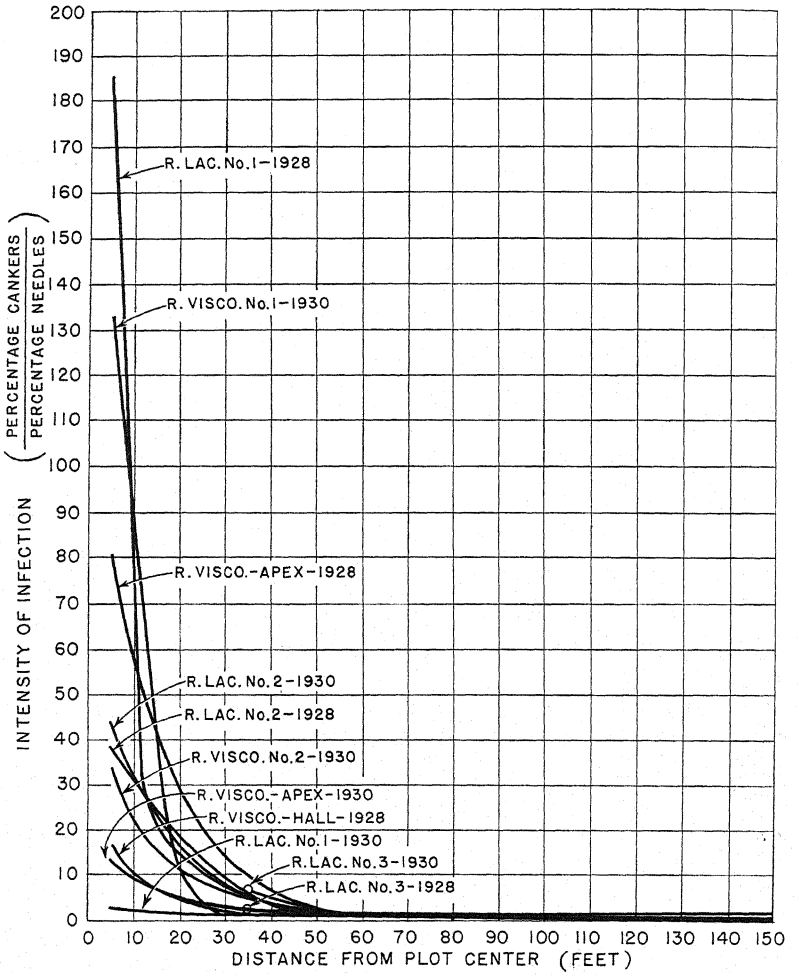


FIGURE 10.—Reduction of pine-infection intensity with increasing distance from ribes at plot centers. The measure of infection intensity consists of the percentage of cankers divided by the percentage of needles in each 10-foot concentric zone out from the center on each plot. Smoothed curves were drawn through the points so derived for each plot. The points are not shown for lack of space but in general fall on or close to their respective curves.

greater damage to pines than from artificial inoculations, even though the latter are purposely made very heavy. It is conceivable, then, for ribes in any white pine stand to be exposed eventually to quantities of aecia sufficient to cause ribes infection and subsequent telia production comparable to that secured on the test plots as shown in table 3.

Under the conditions of ribes infection obtaining in the tests, damaging pine infection resulted from groups of ribes having from 431.0 feet of live stem to as little as 14.4 feet of live stem, which was the smallest amount present on any plot. Such a multiplicity of factors was involved, however, that it was impossible to directly correlate number of feet of ribes live stem with resultant pine infection. Lacustre plot No. 3, with 431.0 feet of *Ribes lacustre* in 1930, had the greatest footage of live stem of any plot. On this plot 17.0 percent of the pines became infected, the highest percentage shown on any plot. For the Hall plot in 1930, directly opposite results were secured, 179.0 feet of *R. viscosissimum* live stem causing not a single canker. The inconsistency is further shown by the results for lacustre plot No. 2 for 1928, on which but 14.4 feet of live stem infected 3.6 percent of the pines, and by the Apex plot in 1928, where but 31.0 feet of *R. viscosissimum* infected 8.1 percent of the pines. The results of these studies, therefore, do not justify the conclusion that a definite footage of live stem of either *R. lacustre* or *R. viscosissimum* will necessarily be responsible for a definite amount of pine infection. The results do justify the conclusion that even a few feet of live stem may and probably will cause appreciable pine infection under conditions comparable to those prevailing on the study areas in British Columbia.

Combining the results from all *Ribes lacustre* tests, it was found that 7.5 percent of the pines within the circular acre became infected from an average of 155.8 feet of ribes live stem in a single year. Similarly, on the *R. viscosissimum* plots 4.5 percent of the pines within the circular acre became infected from an average of 88.5 feet of ribes live stem. If the live stem on the *R. viscosissimum* plots were increased to equal that on the *R. lacustre* plots, then by simple proportion the *R. viscosissimum* plots would show 7.9 percent of the pines infected from that amount of live stem. On this basis equal footages of ribes live stem of the two species might be considered potentially capable of spreading approximately equal amounts of infection to the pines within a circular acre. From these tests it was determined that both ribes species were capable of spreading appreciable infection to pines for a distance of at least 150 feet; and, although the maximum distance of spread in these tests was not determined for *R. lacustre*, it was found that under the conditions existing on the plots *R. viscosissimum* was capable of spreading infection to pines for a distance of about 250 feet. In other studies considerably longer spread has been observed under other conditions, especially with other species of ribes (*11*).

It is pine damage, however, that is of practical importance, rather than ribes infection or pine infection as such. On every test plot and in every year in which pine infection took place as a final result of the inoculation of the central ribes, there occurred appreciable pine damage. As all plots were understocked, the loss of any tree meant actual loss in the board-foot potentialities of the stand. As might be anticipated by those familiar with the rust, percentage damage became less severe with increasing distance from the central ribes (tables 5 and 6). This reduction in damage was solely because of the reduction in intensity of infection with increasing distance from the ribes. The results of these studies showed that in general one out of every four cankers formed on the *Ribes lacustre* plots would probably cause damage, while the proportion was roughly 1 in 5 for the *R. viscosissimum* plots. There is no reason for considering cankers formed from

sporidia produced on *R. lacustre* to be more predisposed to cause damage than cankers initiated by sporidia borne by *R. viscosissimum*. That the ratio of damage cankers to total cankers was higher for plots having *R. lacustre* at their centers than for those with *R. viscosissimum* appears to be largely the result of differences in the character of the pine target exposed.

The ratios of pine damage to pine infection serve to emphasize the fact that under conditions as found on the study plots even relatively light pine infection will result in appreciable damage to the stand. In general, the results showed that this damage would occur within 10 to 15 years following infection of the pines. In these very young stands it does not appear important whether damage occurs in the first year following pine infection or not until the thirtieth year, the pines having no chance to reach merchantability in either case.

Table 5 shows that on the average for all *Ribes lacustre* plots tested 2.9 percent of all pines within the circular acre would suffer sufficient damage, from a single year's ribes infection, to prevent those trees from reaching merchantability. Table 6 shows that 2.3 percent of all trees within the circular acre on the *R. viscosissimum* plots would be similarly damaged. Under infection conditions found on the test plots it is readily apparent that a 15- to 25-percent reduction in stocking could easily occur within a relatively few years. A reduction even within these limits might mean the difference between profit and loss in the management of a young western white pine stand.

It has been shown that the heaviest pine infection and damage occur closest to the ribes. With an amount of ribes equal to that found at the centers of various test plots scattered, as the plants occur in nature, indiscriminately throughout the acre, it would be conservative to estimate damage as being only equal to that secured from a central group.[13] Since fall precipitation during the inoculation years was below average, it appears probable that pine-infection conditions were somewhat less favorable than would ordinarily be expected; this, together with the frequent spread of infection beyond the plot boundaries, indicates that the recorded infection is in no sense to be considered extreme for the given quantities of telia or live stem, but on the contrary must be considered conservative.

SUMMARY

For the purpose of securing more definite data on the capacity of the prickly currant (*Ribes lacustre*) and of the sticky currant (*R. viscosissimum*) to spread damaging white-pine blister rust (*Cronartium ribicola*) infection to western white pine (*Pinus monticola*), so-called ribes-to-pine spread studies were initiated in 1928 in British Columbia, where climatic conditions were reasonably comparable to those in northern Idaho. In these studies, infection was permitted to spread from central groups of artificially inoculated ribes of known footages of live stem to the surrounding stand of otherwise ribes-free white pine reproduction.

Detailed records of telia produced on the central ribes, the pine target exposed to sporidia developed from these telia, and the number and location of cankers formed provided the necessary data for computing the distance and intensity of spread within the plot boundaries

[13] Mielke [8a] has shown how *Ribes lacustre*, scattered naturally throughout a stand, will intensify infection in the pines.

and the resultant damage to pines from each of the two ribes species tested. Climatic data were recorded during test years.

Analysis of these data indicated that under the test conditions equal footages of live stem of the sticky currant and prickly currant are capable of spreading essentially equal infection to nearby white pine reproduction, although no direct and consistent relationship between feet of ribes live stem and resultant pine infection was apparent for either ribes species tested. Within a circular acre, averages from the six tests of *Ribes lacustre* showed that from infection supported by 155.8 feet of live stem 7.5 percent of the pines became infected in a single year. With six tests of *R. viscosissimum*, an average of 88.5 feet of live stem caused infection on 4.5 percent of the pines within a circular acre.

The results further showed that both ribes species were capable of spreading appreciable infection to pines for a distance of at least 150 feet. Under the test conditions *Ribes viscosissimum* spread some infection for a distance of about 250 feet. For both species the intensity of infection was heaviest near the ribes and fell off to relatively low values at a distance of 50 to 60 feet from the central ribes. Percentage of pine damage follows a similar curve of reduction with increasing distance from the ribes. From an average of six tests, 155.8 feet of *R. lacustre* live stem supported infection which spread damage to 2.9 percent of the pines within a circular acre. From an average of six tests, 88.5 feet of *R. viscosissimum* live stem supported infection which spread damage to 2.3 percent of the pines within a circular acre.

Under conditions comparable to those prevailing on the test plots during the years in which the central ribes were inoculated it is possible for as little as 14.4 feet of *Ribes lacustre* live stem to be responsible for damage to 2.8 percent of the white pine reproduction within a circular acre from a single season of ribes infection. Similarly, as little as 31.0 feet of *R. viscosissimum* live stem is potentially capable of supporting infection causing damage, within a single season, to 5.6 percent of the pines within a circular acre.

These studies are significant in indicating that both *Ribes lacustre* and *R. viscosissimum* must be removed from areas that are to be managed for the production of western white pine. Under some conditions, at least, the eradication must be extremely thorough. It is shown that less than 15 feet of ribes live stem can cause damage to stands of white pine reproduction if the ribes bushes are subject to heavy infection, a condition that will exist in areas where sporulating cankers are numerous on associated pines.

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RELATION OF THE CURLY TOP VIRUS TO THE VECTOR, EUTETTIX TENELLUS [1]

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INTRODUCTION

Association of the beet leafhopper, *Eutettix tenellus* (Baker), with a disturbance in beet called "curly leaf" or "blight," now known to have been curly top, was discovered by Ball (*1*)[2] through extensive field observations in Utah. Following this pioneer work, a number of investigators contributed to the knowledge of the relation of the causal agent of the disease to the insect vector.

Shaw (*16*) demonstrated that the feeding of a single leafhopper for only a few minutes was sufficient to initiate curly top on small beets. Smith and Boncquet (*17*) found that a 5-minute feeding period was sufficient for single viruliferous leafhoppers to cause infection.

Boncquet and Hartung (*3*) found that leafhoppers taken from certain natural breeding areas contained no virus, and Stahl and Carsner (*18*) showed that each leafhopper must feed on a plant infected by the virus before it is able to transmit the disease. Severin (*10*) stated that lots of 25 to 50 leafhoppers were able to cause infection in 4 to 6 hours after first feeding on an infected plant. He (*11*) also reported that leafhoppers retained their ability to produce infection throughout their nymphal and adult life, although ability to produce infection decreased with age in the case of leafhoppers transferred daily on beets. Later, however, he (*13*) found that the virus was lost by overwintering females in an average time of 83.9 days.

Carsner and Stahl (*4*) showed that insects caged singly on healthy plants were able to cause infection in 22 hours 45 minutes after having previously fed on infected plants. In experiments carried out by Severin (*12*), individual leafhoppers were able to transmit virus 7 hours after first feeding on an infected plant, and lots of 100 or more were able, in a few instances, to transmit virus in 20 to 30 minutes after first feeding on an infected plant. Nonviruliferous leafhoppers picked up virus in a feeding period of 1 minute, and viruliferous leafhoppers infected healthy plants in a feeding period of 1 minute. Mouth parts were found contaminated with virus after the leafhoppers had fed on diseased plants 30 to 60 minutes. No virus was recovered from feces.

Since curly top transmission is produced consistently only through the agency of the beet leafhopper, a knowledge of the relations of the virus to its insect vector is of considerable importance in the study of other phases of the curly top problem. During the past 6 years, as a part of the curly top investigations conducted at Riverside, Calif., a number of tests have been made, at irregular intervals, to gain more information on this subject. The results of these tests are presented in this paper.

[1] Received for publication Apr. 17, 1937; issued March 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 50

RELATION OF PERIOD OF FEEDING TO ACQUISITION OF VIRUS

Tests were made to determine (1) the minimum time required for leafhoppers to pick up virus from diseased plants and (2) the influence of length of the feeding period on the percentage of fed leafhoppers that became viruliferous. For these tests, adult nonviruliferous leafhoppers were starved for several hours and then caged on diseased beet (*Beta vulgaris* L.) plants for different time intervals ranging from 1 minute to 24 hours. The leafhoppers that fed 1 to 10 minutes, inclusive, were caged singly on diseased beet leaves and watched under a hand lens until they started feeding. The time was noted and the leafhoppers were watched until the expiration of the allotted feeding period, when they were removed and caged on healthy seedling beets for 7 days. Leafhoppers that did not feed continuously during the allotted feeding period were discarded. In feeding periods greater than 10 minutes the leafhoppers were placed in groups of 15 to 20 on diseased plants and left undisturbed during the feeding period. They were then caged singly on seedling beets for 7 days. Leafhoppers reared on diseased plants were used as checks. The feedings on diseased plants were made at temperatures of 95° to 110° F. In this temperature range previously starved leafhoppers usually feed continuously for prolonged periods.

In this experiment leafhoppers picked up virus in a feeding time of 1 minute. The percentage of leafhoppers that acquired virus steadily increased with increase in feeding period up to 5 hours, after which no decided increase was evident up to and including the 24-hour period. However, the check leafhoppers, which were reared to the adult stage on curly top beets, produced more infection than any of the other groups. A summary of the results of these tests is presented in table 1.

TABLE 1.—*Relation of period of feeding of nonviruliferous leafhoppers on curly top plants to acquisition of ability to infect seedling beets*

[150 plants inoculated in each test]

Feeding period (minutes)	Plants infected		Feeding period (hours)	Plants infected	
	Number	*Percent*		*Number*	*Percent*
1	5	3.3	1	66	44.0
2	10	6.7	2	81	54.0
3	13	8.7	3	90	60.0
4	18	12.0	4	93	62.0
5	22	14.7	5	115	76.7
10	23	15.3	6	113	75.3
20	40	26.7	12	110	73.3
30	42	28.0	24	114	76.0
			Check [1]	129	86.0

[1] Leafhoppers reared on curly top plants.

Further tests were made in an attempt to determine the rate at which leafhoppers pick up virus and the feeding time required to obtain a maximum charge. In preparation for this experiment a large number of female nonviruliferous leafhoppers were placed on a healthy beet plant. After 2 days these leafhoppers were removed and the eggs that had been deposited were allowed to hatch. While the nymphs were quite small they were divided into two approximately equal lots. One lot remained on a healthy plant and the other lot was placed on a curly top plant. As soon as all the leafhoppers had

reached the adult stage the nonviruliferous lot was placed on a curly top plant and tested for ability to infect seedling beets and for total virus content at intervals for 3 weeks. Tests were made also of the viruliferous lot, which served as a check on the rate of acquisition of virus by the previously nonviruliferous lot. The two kinds of tests were made as follows: (1) Twenty leafhoppers from each lot were caged singly on seedling beets for 24 hours. The number of plants infected was taken as a relative measure of the ability of the two lots of leafhoppers to transmit virus. (2) The leafhoppers, removed from the seedling beets after 24 hours, were macerated in distilled water and centrifuged, and the supernatant liquid was added to an equal volume of 95-percent alcohol. The resulting precipitate was thrown down by centrifugation, dried, suspended in 1 cc of 5-percent sugar solution, and centrifuged, and the supernatant liquid was reserved for testing. Twenty nonviruliferous leafhoppers were allowed to feed on each test liquid 6 hours, after which they were caged singly on seedling beets 7 days. The number of plants infected was considered a relative measure of the virus content of the two lots of leafhoppers.

The results of this experiment (table 2) indicate that the ability of the test leafhoppers to transmit virus increased rapidly and reached a maximum in 2 to 6 days. During the remainder of the test it was reasonably uniform and approximately equal to that of the viruliferous checks. In contrast to this the virus content of the test leafhoppers increased at a relatively slow rate and reached its maximum on the fourteenth day. Under the conditions of this experiment, the virus content required to enable a leafhopper to reach its maximum efficiency as a vector was considerably less than the maximum charge it is capable of carrying.

TABLE 2.—*Rate of increase in transmissive ability and in virus content of previously nonviruliferous leafhoppers when placed on curly top plants*

Inoculations by means of—	Group of leafhoppers tested [1]	Infection [2] from placing of nonviruliferous leafhoppers on curly top plants for—									
		0 [3] day	1 day	2 days	3 days	4 days	6 days	8 days	10 days	14 days	21 days
		Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Direct feeding, 24 hours.	Originally nonviruliferous.	0.0	32.5	60.0	55.0	67.5	70.0	57.5	62.5	70.0	62.5
	Check	70.0	80.0	62.5	87.5	72.5	70.0	67.5	55.0	52.5	47.5
Extracts from leafhoppers used in tests.	Originally nonviruliferous.	.0	5.0	17.5	15.0	32.5	30.0	22.5	35.0	50.0	45.0
	Check	50.0	47.5	50.0	55.0	50.0	47.5	57.5	72.5	55.0	47.5

[1] The "originally nonviruliferous" leafhoppers were nonviruliferous until placed on curly top beets at the beginning of the experiment; the "check" leafhoppers were reared on curly top plants and remained on curly top plants until tested.
[2] Calculated on the basis of 40 plants that were inoculated in each interval in each test.
[3] Tests made immediately before placing nonviruliferous colonies on curly top beets.

RELATION OF PERIOD OF FEEDING TO INFECTION

In tests of viruliferous leafhoppers to determine the relation of the period of feeding to infection, adult individuals were placed in large cages and starved for several hours. They were then allowed two short feeding periods on healthy beet plants to avoid the effect that starvation has on infection in the first feedings after the starvation

period (see section on effect of fasting period on infection, p. 36). They were then caged singly on cotyledons of seedling beets, watched under a hand lens, and their feeding period recorded. The feedings were made at a temperature of 90° to 110° F., and the feeding periods ranged from ½ to 30 minutes. The tests were repeated at intervals over a period of several months. The plants on which the leafhoppers fed were kept 1 month to determine the number infected.

The results of this series of tests, together with results from 4- and 6-minute tests compiled from other sources, are presented in table 3. No infection was produced in a feeding time of one-half minute and very little in 1 minute. The percentage of infection rose rapidly with increase in the feeding period up to and including the 3-minute feeding period, after which the rate of increase was less.

TABLE 3.—*Relation of period of feeding of viruliferous leafhoppers to infection of seedling beets*

Feeding period (minutes)	Plants inoculated	Plants infected		Feeding period (minutes)	Plants inoculated	Plants infected	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
½	52	0	0.0	6	3,376	1,200	35.5
1	72	1	1.4	10	140	48	34.3
2	343	23	6.7	11 to 15	83	40	48.2
3	159	35	22.0	16 to 20	65	34	52.3
4	87	19	21.8	21 to 25	51	28	54.9
5	567	150	26.5	26 to 30	51	35	68.6

PERIOD OF INCUBATION OF THE VIRUS IN THE LEAFHOPPER

The term "incubation period" as used in this paper refers to the period from the time the leafhopper acquires the virus to the time that it is able to inject enough virus into a plant to produce infection. To determine the length of this period and to gain information regarding the rate of increase in the ability of leafhoppers to cause infection after the acquisition of the virus, an experiment involving a rather extensive series of leafhopper transfers was planned. Young adult leafhoppers, which had been starved 14 to 16 hours, were caged singly and placed on curly top beets at a temperature of approximately 100° F. After 1 hour they were removed and immediately placed on healthy seedling beets. Once each hour for the next 11 hours they were transferred to fresh beets. After the twelfth hour they were transferred to fresh beets at 12-hour intervals for the next 6½ days. Controls were left on diseased beets for 6 days and transferred to healthy plants at 12-hour intervals beginning the seventh day. The last two transfers of the first lot of leafhoppers coincided with the two transfers of the control leafhoppers. Therefore, as a preparation for infecting on the seventh day, the first lot had 1 hour on a diseased plant and eleven 1-hour periods and eleven 12-hour periods on healthy plants; whereas the controls had 6 days on a curly top plant.

Table 4 shows the individual performance of each of 20 leafhoppers on each of the 24 healthy plants exposed to feeding during the 7-day period. The results of nine such experiments are summarized in table 5. Under the conditions of these tests, the minimum incubation period of the virus in the leafhopper was 4 hours. The ability of the leafhoppers to transmit virus increased appreciably during the first 12 hours, but there is no evidence that there was any further increase in infective ability during the following 6½ days.

TABLE 4.—*Infection produced by leafhoppers in successive transfers on seedling beets over a period of 7 days following a 1-hour feeding period on a curly top beet*

Duration and serial No. of feeding period	Plants infected [1] by leafhopper No.—																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1-hour periods:																				
2 [2]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
5	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
6	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
8	−	−	−	+	+	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−
9	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
10	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
12	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
12-hour periods:																				
13	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	+
14	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−
15	+	−	−	+	+	−	−	+	−	−	−	+	−	−	+	+	−	−	+	+
16	+	−	−	+	−	−	−	−	−	−	−	+	−	+	+	−	−	+	+	+
17	−	−	−	+	−	−	−	−	−	−	−	+	+	−	+	−	−	+	+	+
18	+	−	+	+	+	−	+	−	−	−	−	+	−	+	+	+	−	+	+	+
19	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−	+	−	+	+	+
20	+	−	+	−	−	+	−	−	−	−	+	+	−	+	+	+	−	+	+	+
21	−	−	−	+	−	−	−	−	−	+	+	+	−	+	+	−	+	−	−	+
22	−	−	−	+	−	−	−	+	−	+	+	+	+	+	+	−	+	+	+	+
23	−	−	−	+	−	+	+	−	−	−	+	+	+	−	−	−	−	−	−	+
24	+	−	−	+	+	−	−	−	−	−	+	+	+	−	+	+	−	−	+	+
25	−	−	−	+	−	−	−	−	−	+	+	+	−	+	−	−	−	+	+	+

[1] Plus (+) and minus (−) signs indicate diseased and healthy plants respectively.
[2] Feeding period No. 1 was on a curly top beet.

TABLE 5.—*Infection produced by leafhoppers in successive transfers on seedling beets over a period of 7 days following a 1-hour feeding period on a curly top beet* [1]

Duration and serial No. of feeding period	Plants infected										
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6	Experiment 7	Experiment 8	Experiment 9	Total	
1-hour periods:	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	0	0	1	0	1	0	0	5	2.8
5	1	0	1	1	0	0	2	1	2	8	4.4
6	0	1	1	0	0	0	0	2	1	5	2.8
7	3	0	2	1	0	0	0	1	3	10	5.6
8	3	1	1	4	2	1	1	1	5	19	10.6
9	1	1	2	2	3	0	0	3	0	12	6.7
10	2	3	1	2	1	2	1	2	3	17	9.4
11	1	5	0	4	5	1	1	1	1	19	10.6
12	4	8	1	5	1	1	0	1	2	23	12.8
12-hour periods:											
13	5	5	8	6	10	4	3	8	4	53	29.4
14	6	4	8	6	7	8	3	9	4	55	30.6
15	2	4	5	4	3	4	3	8	10	43	23.9
16	7	5	8	6	7	10	3	9	9	64	35.6
17	4	4	5	4	8	6	4	7	7	49	27.2
18	6	6	8	6	5	11	1	8	13	64	35.6
19	9	3	9	3	8	8	2	9	7	58	32.2
20	-----	-----	7	5	7	7	1	8	11	46	32.9
21	-----	-----	6	3	6	8	1	6	9	39	27.9
22	-----	-----	7	4	3	7	2	10	13	46	32.9
23	-----	-----	4	4	6	3	2	6	7	32	22.9
24	-----	-----	7	5	7	4	4	6	10	43	30.7
25	-----	-----	7	7	11	3	2	3	7	40	28.6
Checks: [2]											
24	-----	-----	7	14	14	5	8	7	11	66	47.1
25	-----	-----	6	16	13	0	8	5	5	53	37.9

[1] 20 leafhoppers were used in each experiment.
[2] These leafhoppers fed on curly top beets over a period of 6 days and were then transferred to seedling beets at 12-hour intervals.

In each of the nine experiments there was a striking variation in the amount of infection produced by the different individuals of the group. This is illustrated in table 4. Leafhopper No. 4 produced infection in the fifth hour, failed to produce infection in only two of the remaining 1-hour feeding periods, and failed to produce infection in only three of the 12-hour feeding periods; whereas leafhopper No. 6 infected only two plants in 7 days and these were in the 12-hour feeding periods. This type of performance is apparently the result chiefly of characteristics of individual leafhoppers and is not due necessarily to a variation in the amount of virus originally picked up by the leafhoppers, since similar variations are obtained when leafhoppers reared on curly top plants are used in successive short-interval transfers.

Whether the slightly greater amount of infection by the check group of leafhoppers on the seventh day is of significance is difficult to determine. A factor which cannot be evaluated and which may have influenced results is the relative number of leafhoppers in the two groups that actually carried virus. Of the 140 leafhoppers used in the frequent transfers, 32 produced no infection. If it were assumed that these 32 obtained no virus in the 1-hour period of feeding on diseased beets and that all the leafhoppers allowed to feed on diseased beets for 6 days obtained virus, part of the difference in infection might be accounted for. Even without any correction for this factor, it is evident from the totals shown in table 5 that the check leafhoppers were superior only to a small degree to those of the frequently transferred lot in ability to infect on the seventh day.

Evidence as to the relative amounts of virus carried by the two lots of leafhoppers at the end of 7 days was obtained in four of the nine tests by removing each lot of 20 leafhoppers and testing for virus content by the alcoholic-precipitation method already described. Five of the eighty plants inoculated from the material derived from the frequently transferred leafhoppers were infected; whereas 24 of the 80 plants inoculated from material derived from the control leafhoppers were infected. This is strong evidence of a greater virus content in the control group, and it shows that ability to infect is not always proportional to the amount of virus carried in the leafhoppers.

EFFECT OF LENGTH OF FASTING PERIOD ON INFECTION IN SHORT-TIME FEEDINGS

The effect of a period of fasting on ability of leafhoppers to produce infection was noted in a series of tests made in connection with the study of variation in infective ability discussed in a later section. The viruliferous leafhoppers to be tested were starved overnight. Each leafhopper was then allowed to feed 6 minutes on each of 20 seedling beet plants as rapidly as it would complete its feedings. The beginning and ending of each feeding period were recorded, thus making available a complete record of the feeding time and the fasting interval for each of the 20 feedings of each leafhopper. The records of 209 leafhoppers are available from these experiments.

When all the feeding tests were completed, the data were assembled on the basis of the percentage of infection produced in each of the 20 successive feedings. As shown in figure 1, where these data are plotted, infection was relatively low in the first feeding period, some-

what greater in the second, and rose in the third to a level that was maintained rather consistently for the succeeding 17 periods.

The percentage of infection was calculated also on the basis of the length of the period of fasting prior to feeding, in an effort to determine the effect of fasting periods of different duration on infection in the subsequent 6-minute feeding. The fasting periods varied from less than 1 minute to more than 18 hours. The data for a total of 4,045 fasting periods were arranged in 21 groups, as follows: 10 groups with fasting periods increasing by 1-minute intervals, respectively, from 1 to 10 minutes; 4 groups with fasting periods increasing by 5-minute intervals, respectively, from 15 to 30 minutes; 6 groups with fasting periods increasing by 1-hour intervals, respectively, from 1 to 6 hours; 1 group with a fasting period of 18 hours.

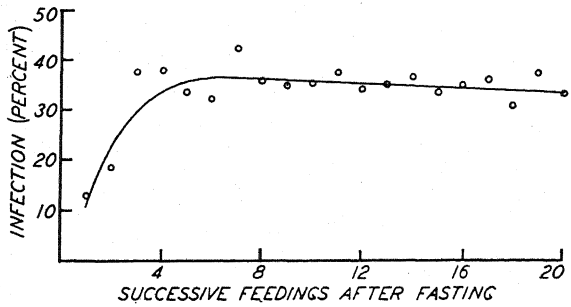


FIGURE 1.—Infection of seedling beets caused by viruliferous leafhoppers in 20 successive 6-minute feedings following an initial 16- to 20-hour fast period.

The results are plotted in figure 2, the percentages of infection being shown on the ordinate axis. These points represent averages of infections based on from 4 to a maximum of 475 cases. Fasting period is plotted on the abscissa axis as the logarithm of the time in minutes. There is some indication from the data that when the leafhoppers fast only 1 to 3 minutes before feeding they are less effective vectors than when they fast 15 minutes to 3 hours before feeding. Infections secured following an 18-hour fasting period were extremely low.

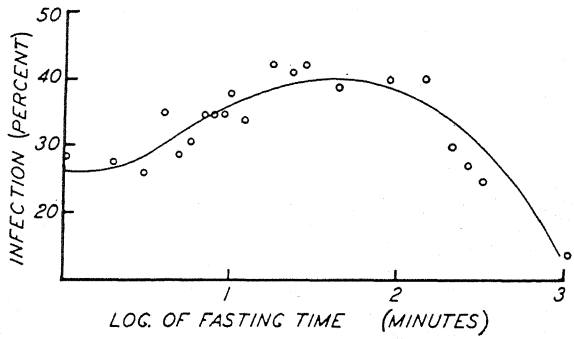


FIGURE 2.—Effect of length of the fasting period immediately before inoculations were made with viruliferous beet leafhoppers on the infections secured with seedling sugar beets in a subsequent 6-minute feeding period.

Little is known regarding the factors that produce these results. The failure of leafhoppers to produce the normal amount of infection in the first and second 6-minute feedings after a long fasting period can hardly have been due to a weakened condition of the leafhoppers, for they were quite active and apparently in good physical condition. The manner in which they fed varied in no way from that in later feedings, so far as known. It is suggested, however, that starvation,

combined with a certain amount of desiccation, may have brought about certain changes that tended to inactivate any virus that might have been held in the salivary glands; hence the usual amount of infection could have been produced only after these conditions were corrected.

The greater amount of infection produced in the feedings that followed fasting periods of 15 minutes to 3 hours may have resulted from the injection of greater amounts of saliva into the plant in feedings following the fast, or it may have come from an increase in virus content of the salivary glands by accretion from the blood during the fast, or it may have been due to other factors. However, an increase in virus content of the salivary glands by virus multiplication during the fast would seem to be excluded in view of evidence presented later in this paper which indicates that no multiplication of virus occurs in the leafhopper.

DISTRIBUTION OF VIRUS IN DIFFERENT PARTS OF THE LEAFHOPPER

The determination of the distribution and location of the virus in the beet leafhopper was only partially accomplished, because of the difficulty of avoiding contamination from parts other than those selected for test. Tests to gain some concept of the virus content of the blood, salivary glands, alimentary tract, and feces were made as follows:

Blood.—Leafhoppers were etherized and fastened to a piece of cardboard by means of a thread drawn tight enough to place a slight pressure on the thorax. Under a dissecting microscope, a small opening was made in the abdominal wall, through which a small drop of liquid escaped. This coagulated in a short time and was removed on the point of a needle to a 5-percent sugar solution. Drops of blood from 20 leafhoppers were mixed with 1 cc of sugar solution, and 20 nonviruliferous leafhoppers were allowed to feed on the mixture. The leafhoppers were then caged singly on seedling beets to determine whether they had picked up virus. A wash of alcoholic precipitate of the macerated remains of the leafhoppers from which the drops of blood were taken was tested for virus and a further test was made of the virus content of 20 viruliferous leafhoppers from which no blood was removed.

Salivary glands.—The heads with part of the thorax were removed from 20 viruliferous leafhoppers. The salivary glands were dissected out in drops of water, passed through distilled water, and macerated in 1 cc of sugar solution, and 20 nonviruliferous leafhoppers were allowed to feed on the mixture. As a check, the remains of the dissected leafhoppers and also 20 nondissected leafhoppers were tested by the alcoholic-precipitation method.

Feces.—Large numbers (200 to 300) of viruliferous leafhoppers were placed on curly top diseased leaves in large Erlenmeyer flasks and allowed to feed at a temperature of 85° to 110° F. for 3 to 4 hours. Air was circulated to keep the flasks dry. The feces deposited on the sides of the flasks were removed by washing with distilled water. Alcohol was added and a wash of the precipitate was prepared and tested for virus. An extract from 20 viruliferous leafhoppers was tested as a check.

Alimentary canal.—Viruliferous leafhoppers were caged on petioles of healthy beets for 24 hours in order to allow for partial or complete removal of materials previously taken up from diseased plants. After this period the alimentary tracts from 20 leafhoppers were dissected out in drops of distilled water. The alimentary tracts were then passed through distilled water and macerated in sugar solution. The Malpighian tubules were included with the tested parts, and undoubtedly some blood was carried over. The remains of the dissected leafhoppers and also 20 nondissected leafhoppers were tested as checks.

The results of these tests (table 6) show that virus was obtained from all of the parts tested. Probably the results from blood and feces may be considered the most reliable, since the blood and feces had least chance of being contaminated with virus from other parts. These results indicate that a small amount of virus passed through the leafhopper and was present in active condition in the feces. There is apparently much less virus in the salivary glands than in the remainder of the leafhopper. This seems to show that the salivary glands are not the virus reservoirs of the insect.

TABLE 6.—*Virus content of different parts of the beet leafhopper*

Experiment No.	Source of inoculum	Plants inoculated	Plants infected	
		Number	*Number*	*Percent*
1	Blood	60	14	23.3
	Leafhoppers (remains)	60	21	35.0
	Leafhoppers	60	23	38.3
2	Salivary glands	40	10	25.0
	Leafhoppers (remains)	40	28	70.0
	Leafhoppers	40	26	65.0
3	Feces	148	18	12.2
	Leafhoppers	140	87	62.1
	Alimentary canal	40	18	45.0
4	Leafhoppers (remains)	40	22	55.0
	Leafhoppers	40	17	42.5

DOES THE VIRUS MULTIPLY IN THE INSECT VECTOR?[3]

The manner of transmission of plant viruses by insects undoubtedly varies, depending on the species of insect involved and perhaps on the virus also. Certain insects transmit apparently by simple transfer of the virus on the mouth parts. In such cases it is probably unnecessary for the virus to pass through the body of the insect. Vectors of this type soon lose their power to cause infection when deprived of new sources of virus.

Other insects ingest virus and retain it in their bodies over more or less extended periods, as is shown by their ability to transmit disease after being confined to immune plants. Retention of virus in this way has led to the suggestion of a biological relation between virus and insect in certain instances. That this relationship involves a stage of development of the virus in the insect has not been shown, but it has been rather generally accepted as evidence that certain viruses multiply in their insect vectors.

Kunkel (*9*) suggested that the long incubation period of the virus of aster yellows in the vector *Cicadula sexnotata* Fall and the length

[3] After this paper was prepared for publication, Freitag (*6*) published an article in which extensive evidence is presented indicating that the curly top virus does not multiply in the insect vector.

of time it retains the virus when confined to immune plants indicate multiplication of the virus in some tissue of the insect.

In studies on *Cicadulina mbila* Naude, vector of the virus of streak disease of maize, Storey (*19*) developed "active" races of the insect, capable of causing infection after feeding on diseased plants, and "inactive" races, incapable of causing infection after feeding on diseased plants. When transferred to virus-free plants at frequent intervals, active individuals usually retained the virus during life but certain individuals lost it. Leafhoppers that had lost the virus regained their ability to produce infection after feeding on diseased plants. Inactive individuals were able to act as vectors when virus was introduced into their body cavities by artificial means. However, these leafhoppers retained their ability to transmit for only a relatively short period. Apparently the size of the initial charge of virus greatly influenced the length of time in which the leafhopper could produce infection. Therefore, it seems probable in this case that there was no multiplication of virus in the insect.

Fukushi (*8*) found that the virus of dwarf disease of rice is carried through the egg stage to the third generation in the leafhopper *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl. This probably is the strongest evidence available that a plant virus multiplies in its insect vector, for in the absence of multiplication infection from enormous dilutions would be required.

Severin (*11*) found that male beet leafhoppers when transferred daily to healthy sugar beets retained their ability to produce infection throughout their adult life. Other evidence supports the view that after leafhoppers acquire the virus of curly top they retain their ability to produce infection for long periods under a wide range of conditions.

A number of experiments were performed to determine whether this ability is due to multiplication of the virus in the insect or to a long period of retention of the virus acquired by the insect through feeding.

In one series of tests attempts were made to introduce the virus into the blood of the leafhopper in a manner similar to that employed by Storey (*20*) in introducing the virus of maize streak into *Cicadulina mbila*. In these tests nonviruliferous leafhoppers were etherized and bound to a piece of cardboard under a binocular microscope. With the aid of a micromanipulator, the tip of a capillary tube filled with a medium containing the curly top virus was inserted into the abdominal cavity and a portion of the liquid injected into the leafhopper. Fifty leafhoppers were treated in this manner and caged on seedling beets. Mortality among the leafhoppers was high and after 7 days only seven were alive. No infection was obtained with the seedling beets.

In other tests, punctures through drops of media containing the virus were made in the abdominal and thoracic cavities of nonviruliferous leafhoppers by means of small needles. Phloem exudate from diseased beets and drops of blood from viruliferous leafhoppers were used as sources of virus. One hundred leafhoppers were treated and caged singly on seedling beets. Mortality among these leafhoppers was much lower than among those injected by means of capillary tubes. After 7 days, 51 leafhoppers were alive. No infection was obtained on the test beets.

To determine whether the virus content of the leafhoppers decreased with feeding on plants from which they could obtain no additional

virus, viruliferous leafhoppers were confined to plants very resistant or immune to curly top and tested at intervals for ability to infect seedling beets and for virus content.

In the earlier tests, Australian saltbush (*Atriplex semibaccata* R. Br.) was selected as the resistant plant on which to confine the leafhoppers. Although extremely resistant, this plant is probably not immune to curly top under all conditions, for Severin and Henderson (*15*) succeeded in obtaining a small amount of virus from 3 of 30 plants previously inoculated repeatedly by means of large numbers of viruliferous leafhoppers. In all tests reported here, large numbers of the nymphal progeny of the colonies on Australian saltbush were caged on seedling beets at intervals during the course of the test to determine whether they had acquired virus. None produced infection, and it was accordingly assumed that the adult leafhoppers of the colonies had not acquired virus from the plant. After Severin and Freitag (*14*) showed that sweet corn is a favorable food plant for the leafhopper and also immune to curly top, all experiments were repeated with the Golden Bantam variety of sweet corn used in place of Australian saltbush. No significant difference was obtained with leafhoppers confined on the two species, and it seems probable that the results obtained with Australian saltbush are as reliable as those obtained with corn.

Three tests were made. In the first test, colonies of viruliferous leafhoppers reared to the adult stage on curly top beets were divided into two lots. One lot remained on curly top beets and the other lot was transferred to Australian saltbush or sweet corn. Tests of the ability to produce infection were made at weekly intervals by caging, singly, 20 leafhoppers on seedling beets for 24 hours. After 24 hours, the 20 leafhoppers of each lot were macerated and tested for virus content by the alcoholic-precipitation method already described. The results are summarized in experiment 1 of table 7.

In the second test, colonies of approximately 1,000 nonviruliferous leafhoppers just emerged into the adult stage were placed on curly top plants for a period of 2 hours at a temperature of about 110° F. This short feeding period was used in order to provide a smaller charge of virus than that held by leafhoppers reared on diseased plants. At the expiration of the 2-hour feeding period each colony was divided into two lots. One lot was placed on Australian saltbush or sweet corn and the other lot remained on curly top beets. At intervals, 20 leafhoppers were taken from each lot and caged singly on seedling beets for 24 hours. The leafhoppers were then macerated and each lot tested for virus content as in the first experiment. The results are summarized in experiment 2 of table 7.

In the third test nonviruliferous leafhoppers were allowed a feeding period of 6 hours on a water wash of an alcoholic precipitate from macerated viruliferous leafhoppers. Previous tests had shown that leafhoppers acquire very low charges of virus through feeding on such suspensions. After feeding on the liquid the leafhoppers were caged on sweet corn. At intervals, 20 leafhoppers were removed and caged 7 days on seedling beets to determine their ability to infect. At the same time 20 other leafhoppers were removed from the cage and tested for virus content by the alcoholic-precipitation method already described. Checks were run with leafhoppers from curly top beets as in other experiments. The results of several repetitions of this experi-

TABLE 7.—*Influence of size of initial virus charge on the decrease in infective ability and virus content of leafhoppers deprived of new sources of virus by being confined to resistant or immune plants*

Experiment No.	Way in which leafhoppers acquired virus	Inoculations[1] on seedling beets by means of—	Plants on which leafhoppers were kept until tested on seedling beets	Infection[2] on seedling beets after—										
				0 week[3]	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks
				Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	4 weeks feeding on curly top beets.	Direct feeding, 24 hours	Saltbush or corn	62	67	52	63	62	55	51	52	72	-------	-------
			Curly top beet	76	63	73	71	73	76	83	50	80	-------	-------
		Extracts from leafhoppers used in test.	Saltbush or corn	45	38	13	3	5	12	11	2	5	-------	-------
			Curly top beet	43	46	68	68	66	67	66	60	60	-------	-------
2	2 hours feeding on curly top beets.	Direct feeding 24 hours	Saltbush or corn	32	67	76	46	45	38	28	32	21	25	-------
			Curly top beet	36	53	61	68	71	51	50	52	48	70	-------
		Extracts from leafhoppers used in test.	Saltbush or corn	21	26	3	5	3	6	0	7	2	0	-------
			Curly top beet	21	45	28	42	68	45	30	42	47	55	-------
3	6 hours feeding on extracts from viruliferous leafhoppers.	Direct feeding, 7 days	Saltbush or corn	43	-------	29	-------	14	-------	7	-------	2	-------	0
			Curly top beet	38	-------	91	-------	96	-------	89	-------	93	-------	86
		Extracts from leafhoppers from the same sources as those used in test.	Saltbush or corn	1	-------	0	-------	0	-------	0	-------	0	-------	0
			Curly top beet	0	-------	42	-------	36	-------	31	-------	47	-------	32

[1] Leafhoppers were caged singly on seedling beets 24 hours in experiments 1 and 2. The resulting infection is considered a relative measure of their ability to infect. An extract from these leafhoppers was then prepared and fed to nonviruliferous leafhoppers. These were caged singly on seedling beets 7 days. The resulting infection is considered a relative measure of the virus content of the leafhoppers from which the extract was derived.

[2] In experiments 1 and 2, 100 plants were inoculated in each test at each time interval. In experiment 3 the number of inoculated plants per test ranged from 40 to 180.

[3] These tests were made immediately following the feeding period in which the test leafhoppers acquired their charge of virus.

An additional test was made in which leafhoppers were transferred at daily intervals to healthy beets. In this test 28 half-grown non-viruliferous nymphs were allowed to feed on a virus suspension, and 6 hours later they were caged singly on healthy beets having four to six leaves. The leafhoppers were transferred daily to fresh beets over a period of 90 days or until they died. Infection decreased with time and no infection was produced by any of these leafhoppers after 54 days. The record of five leafhoppers for 90 days is shown in table 8. On the ninety-first day these leafhoppers were allowed to feed on a curly top plant and were then transferred daily to healthy beets over a period of 9 days. Three of these leafhoppers acquired virus and infected a large percentage of the plants on which they fed. This proves conclusively that these leafhoppers had not lost their ability to act as vectors and that their previous inability to infect was due to depletion of their virus content.

TABLE 8.—*Plants infected*[1] *in 90 successive days by five leafhoppers that had previously acquired a low charge of virus by feeding 6 hours on extracts from viruliferous leafhoppers; also plants infected by the same leafhoppers after feeding 1 day on a curly top plant*

Day No.	Infection by leafhopper No.—					Day No.	Infection by leafhopper No. —				
	1	2	3	4	5		1	2	3	4	5
1	+	−	+	−	+	30	−	−	−	−	+
2	−	−	+	−	+	31-33	(2)	(2)	(2)	(2)	(2)
3	−	−	+	−	+	34	+	+	+	−	+
4	−	−	−	−	+	35	+	−	−	−	−
5	−	−	−	−	−	36	+	−	−	−	−
6	−	−	−	−	+	37	−	−	−	−	−
7	+	−	−	−	+	38	−	−	−	−	+
8	+	−	−	−	+	39	−	−	+	−	−
9	−	−	−	−	+	40	−	−	−	−	+
10	−	−	+	−	+	41	−	−	−	−	+
11	−	−	+	−	+	42	−	−	−	−	−
12	−	−	−	−	+	43	−	−	−	−	+
13	−	−	+	−	+	44	−	−	−	−	−
14	−	−	+	−	+	45	−	−	+	−	+
15	−	−	−	−	−	46	−	−	−	−	+
16	−	−	−	−	+	47-52	(2)	(2)	(2)	(2)	(2)
17	−	−	−	−	−	53	−	+	−	−	−
18	−	−	+	−	+	54	−	−	−	−	+
19	−	+	−	−	+	55-90	(2)	(2)	(2)	(2)	(2)
20	−	−	−	−	−	91	(3)	(3)	(3)	(3)	(3)
21	−	−	−	−	+	92	+	−	−	−	+
22	−	−	−	−	+	93	+	−	+	−	+
23	−	−	−	−	+	94	+	−	+	−	+
24	−	−	−	−	+	95	+	−	+	−	+
25	−	−	−	−	−	96	+	−	+	−	+
26	−	−	−	−	−	97	+	−	−	−	−
27	−	−	−	−	+	98	−	−	+	−	+
28	−	−	−	−	+	99	+	−	−	−	+
29	−	−	+	−	+	100	+	−	+	−	+

[1] Plus (+) and minus (−) indicate diseased and healthy plants, respectively.
[2] No plants were infected on these days.
[3] The leafhoppers fed on a curly top plant during this day.

The results of these experiments showed that when leafhoppers were transferred at frequent intervals to beets or were confined to plants extremely resistant or immune to curly top their virus content decreased with time. The decrease in virus content was more rapid than the decrease in ability to produce infection by direct feeding, which is further evidence that the amount of virus required by the leafhopper for maximum ability to infect is appreciably less than the maximum charge of virus that it is capable of carrying. The level

to which the virus content and ability to infect dropped in 8 to 10 weeks was influenced by the size of the charge of virus carried by the leafhoppers at the time they were placed on immune plants.

In view of the foregoing evidence and the failure to impart ability to infect by injecting virus suspensions into the leafhoppers, it seems probable that there is no multiplication of the curly top virus in the beet leafhopper. The prolonged periods over which the leafhopper is able to produce infection when deprived of plant sources of virus may be explained by the ability of the virus to retain its activity in the body of the leafhopper for long periods and to a slow rate of loss of virus by the leafhopper. The period of virus retention is surprisingly long.

INDIVIDUAL VARIATION IN ABILITY TO TRANSMIT THE VIRUS

From results already presented it is evident that individual beet leafhoppers vary greatly in their ability to transmit the curly top virus. Similar individual variations in infective ability have been found in at least two other vectors of plant viruses.

Storey (*19*) found individuals of *Cicadulina mbila* incapable of transmitting the virus of streak disease of maize under the conditions of his experiments. Selection and mating in successive generations produced races in which none of the individuals were capable of transmitting virus. Selection and mating also gave races in which all the individuals were able to transmit virus. Tests of progeny of crosses between the two races indicated that ability to transmit is a sex-linked dominant character. Fukushi (*7*) observed a wide variation in the ability of *Nephotettix apicalis* var. *cincticeps* to transmit the virus of stunt disease of rice and found numerous individuals apparently unable to transmit it even after they had fed throughout their nymphal stages on infected plants.

By testing large numbers of beet leafhoppers from stock colonies and field collections individuals were found that failed to transmit the curly top virus to small beets in 7 to 14 days in sufficient quantities to cause infection. However, when successive selections and matings were made from these leafhoppers their F_3, F_5, and F_7 progeny retained the ability to transmit virus when caged 14 days on seedling beets. These tests were extensive enough to indicate strongly that, in the colonies used, there were no individuals inherently lacking in ability to transmit the virus. It was not determined whether these selections were less effective than the original stock in transmitting virus in short-interval transfers.

An attempt was made by successive selection and mating to develop strains of leafhoppers that would differ in ability to transmit the virus. In these tests individuals were allowed to feed 6 minutes successively on each of 20 seedling beets. The percentage of infection resulting was considered a measure of the transmissive ability of the leafhopper. Individuals that produced little or no infection in these tests were mated, and selections were made from the progeny in each successive generation through the F_7 generation. A similar process of breeding and selection was carried through the F_6 generation for leafhoppers high in ability to transmit. At first selections were made of the most promising individuals regardless of source, but later close inbreeding in each line was practiced and only brother and sister matings were

made. Tests of the progeny of each generation were made as described, except in the F_5, F_6, and F_7 generations of the low strain. Here, in order to give the leafhoppers a greater chance to produce infection, they were left on the seedling beets 12 to 24 hours.

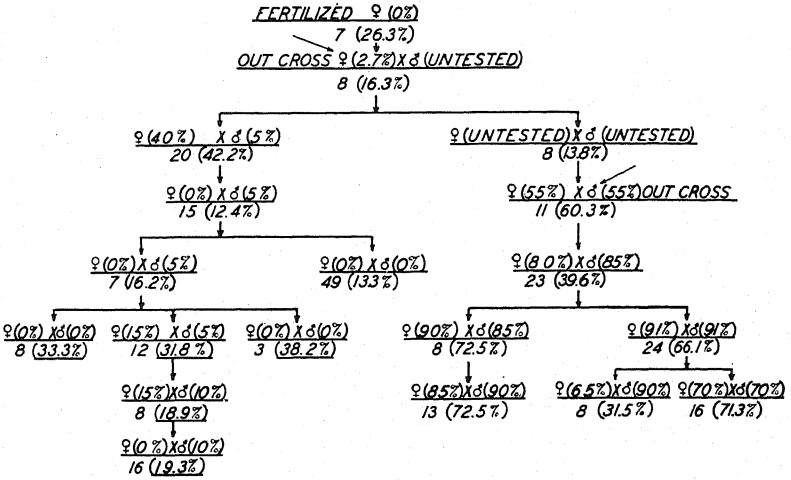


FIGURE 3.—Results of selecting and mating beet leafhoppers differing in ability to transmit curly top virus. In the diagram the percentage shown above the line following the symbol for sex shows the infective capacity found. The first number below the line indicates the number of leafhoppers in the progeny of this mating that were tested. The second number (in parentheses) indicates the average percentage of infection produced. Percentages of infection were determined by the number of plants infected when 20 plants were inoculated. Six-minute feedings were used except in the tests of progenies of the last three generations of the low strain (numbers underlined), in which the feeding periods were longer (12 to 24 hours).

These tests were not carried as far as was desirable and the original selections were not as rigid as they should have been, especially in the strain selected for high ability to transmit. The two lines of descent, including the individuals that were mated and the results of the tests of the progeny of each mating, are shown in figure 3.

Figure 4 shows a graphic analysis of the entire tested populations of each strain from the third generation to the end of the selections, based on the number of plants each leafhopper infected out of the 20 plants on which it fed. Figure 4 includes all the leafhoppers from figure 3 and several more from each strain which for various reasons were not included in figure 3. The data in figure 4 show that the leafhoppers of the low strain were predominantly low in ability to transmit and that the leafhoppers of the high strain were predominantly high in ability to transmit.

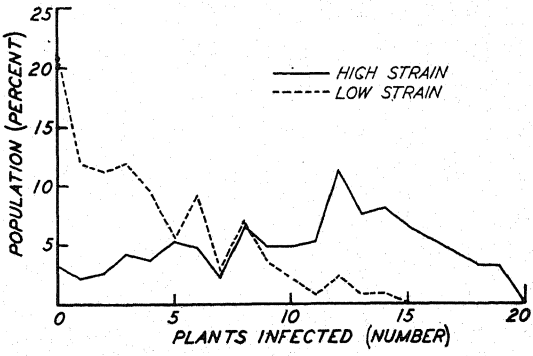


FIGURE 4.—Distribution of the total tested populations of the high and low strains (beginning with the third generation) on the basis of individual ability of leafhoppers to transmit curly top virus as indicated by the number of plants infected by each individual in the tests in which each leafhopper fed successively on each of 20 seedling beets.

The individuals of two populations shown in figure 4 were divided into two groups. Those individuals that infected seven plants or less were considered low in ability to transmit and those that infected eight plants or more were considered high in ability to transmit. On this basis, 119 of the 143 tested individuals of the low strain were low in ability to transmit and 24 individuals were high. In the high strain, 53 of the 186 individuals were low in ability to transmit and 133 were high.

Using these data to determine χ^2 according to Fisher's (*5*) method for testing for independence in a 2×2 classification, a value of 97.038 was obtained. Since a value of 6.635 gives odds of 100 to 1 favoring independence, a χ^2 value of 97.038 would give very high odds in favor of the concept that the two strains of leafhoppers used in these experiments were genetically different.

The foregoing results seem to indicate clearly that degree of effectiveness in transmitting curly top virus is heritable and that strains of leafhoppers below the average and strains above the average in transmissive ability can be developed. As yet, however, there is no evidence that a strain inherently lacking in ability to transmit the curly top virus can be developed.

RELATION OF CURLY TOP VIRUS TO CERTAIN NONVECTOR INSECTS

The only vector of the curly top virus in North America is the leafhopper *Eutettix tenellus*. None of the other species of insects that feed on sugar beets or other plants susceptible to curly top have been found capable of transmitting the virus, even when used in very large numbers. Several of the species of insects that feed on beets seek out the phloem and must of necessity pick up virus in the course of their feeding since the virus content of the phloem is high (*2*). The relations of of the virus to these insects and the factors preventing their acting as vectors hold considerable interest for those dealing with virus diseases.

ACQUISITION OF VIRUS

A number of the common species of insects that feed on sugar beets were tested for virus content after being allowed to feed on curly top beets. These species [4] include the aphids *Myzus persicae* (Sulz.), *Aphis rumicis* L., and *Pemphigus betae* Doane; the leafhoppers *Aceratagallia californica* (Baker), *Phlepsius strobi* (Fitch), and *Empoasca solana* (De Long); the thrips *Hercothrips femoralis* (Reuter); and a red spider, *Tetranychus* sp. Freehand and prepared sections of beet leaves have demonstrated that *Aphis persicae* and *Aceratagallia californica* are phloem feeders. Probably *Aphis rumicis*, *Pemphigus betae*, and *Phlepsius strobi* also feed on the phloem. The remaining three species probably obtain all or most of their food from the parenchyma.

The insects were caged on diseased beets 2 days to 2 weeks, the time varying with the species of insect involved. At the expiration of the feeding period, a 30-mg sample of insects was taken. An extract was prepared from this in the manner already described, and

[4] The writers are indebted to P. W. Oman for identification of *Empoasca solana* and *Aceratagallia californica;* to J. C. Crawford and Stanley F. Bailey for identification of *Hercothrips femoralis;* to H. E. Ewing for identification of *Tetranychus* sp.; and to P. W. Mason for identification of *Myzus persicae.*

fed to nonviruliferous beet leafhoppers. After a feeding period of 4 to 6 hours on the prepared extract, the leafhoppers were caged singly on seedling beets for 7 days. The number of plants infected was taken as a measure of the virus content of the insects from which the extracts were derived. The virus content of the leaves of the diseased beets on which the insects had fed was tested in the usual way by the alcoholic-precipitation method, 1 cc of juice being used in each test.

All the species of insects used in these tests picked up virus from diseased beets, and some of them acquired it in relatively large quantities. One might judge that the virus per unit weight is more concentrated in some of these insects than in the beet juice. It is worthy of note that those species that presumably fed only on the parenchyma acquired virus, although apparently the quantity was much smaller than that obtained by insects that fed on the phloem. No method has been devised for determining the original tissue source of the virus picked up by insects that restrict their feeding to parenchyma. While it may have been picked up directly from the parenchyma cells, it seems more probable that it was derived from phloem exudate that had escaped into the intercellular spaces of the parenchymatous tissues. The results of these tests are shown in table 9.

TABLE 9.—*Acquisition of virus by different species of insects*

Insect tested	Source of virus and results					
	Extracts from macerated insects			Extracts from juice of plants on which insects fed		
	Plants inoculated	Plants infected		Plants inoculated	Plants infected	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Aceratagallia californica	140	62	44.3	100	42	42.0
Phlepsius strobi	20	5	25.0			
Myzus persicae	100	61	61.0	20	15	75.0
Aphis rumicis	100	23	23.0	100	31	31.0
Pemphigus betae	60	9	15.0	20	12	60.0
Empoasca solana	180	37	20.5	180	69	38.3
Hercothrips femoralis	220	22	10.0	160	70	43.8
Tetranychus sp. (from beet)	60	17	28.3	60	28	46.7
Tetranychus sp. (from oxalis)	80	11	13.8	80	0	.0

LONGEVITY OF VIRUS IN NONVECTOR SPECIES

With the exception of the aphid *Pemphigus betae,* the species of insects mentioned above were used in tests to determine the time the virus remained active in the insects after they were removed from diseased plants. Large numbers of individuals of each species were caged on curly top beets for 2 days to 2 weeks, after which they were transferred to healthy beet plants. At intervals, 30-mg quantities were removed and tested for virus content. The two species of aphids multiplied so rapidly that it was difficult to keep the individuals that had fed on diseased plants separate from their progeny. After a few preliminary trials, attempts to effect this separation were abandoned and tests were made on 30-mg samples from each colony without regard to age of insects used. With all other species, only the individuals that fed on diseased plants entered into the tests for the presence of virus.

Myzus persicae retained virus 14 days and *Aphis rumicis* retained it 7 days. It is probable that virus could have been recovered from these two species after longer periods on healthy plants if tests had been restricted to the insects that fed on diseased plants. *Acerata-gallia californica* retained virus 21 days, though there was a progressive drop in virus content from the time the leafhoppers were removed from diseased plants. Those species that fed only on parenchyma lost the virus in a relatively short time. The results of these tests are shown in table 10.

TABLE 10.—*Retention of virus by different species of insects*

Insect tested	Plants inoculated	Plants infected from extracts of insects after the insects had been on healthy beet plants for—					
		0 days [1]	1 day	3 days	7 days	14 days	21 days
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Aceratagallia californica	80	35	21	18	9	2	1
Phlepsius strobi	20	8	0	0	0	----	----
Myzus persicae	40	24	22	20	9	2	0
Aphis rumicis	100	25	22	16	11	0	----
Empoasca solana	80	22	10	0	0	----	----
Hercothrips femoralis	20	1	0	0	0	----	----
Tetranychus sp	60	5	2	1	0	----	----

[1] Tests made immediately after removal of the insects from curly top plants.

DISCUSSION

The curly top virus extracted from the plant by the beet leafhopper in its feeding is probably derived chiefly from the phloem. As the leafhopper feeds, the virus evidently passes through the mouth parts into the alimentary canal along with food materials. Little is known regarding the effect of the contents of the alimentary canal on the virus. The experiments reported here show that the virus passed through the insect and was recovered in the feces. The concentration of virus in the feces could not be determined, but there is reason to suspect that it was far below that of the food material that the leafhopper ingested. Experiments by Severin (*12*) indicate that under certain conditions no virus is recoverable from the feces. It seems probable, therefore, that there may be considerable inactivation of virus in the alimentary tract. Studies with dyes indicate that at high temperatures ingested materials pass through the leafhoppers in 30 minutes to 2 hours. Therefore, if there is inactivation of virus in the alimentary tract, the inactivating substances must be quite effective and capable of acting in a relatively short time.

The virus passes from the food material taken in by the leafhopper, through the walls of the alimentary canal, into the blood, where, according to present indications, it reaches a maximum concentration after the leafhopper has fed on diseased plants for 7 days or more.

The blood would appear to be a very favorable medium for preserving the virus. In reaction it is slightly alkaline (*2*), in this respect being somewhat similar to phloem content, which is considered the most favorable plant medium for the virus. The high colloidal content of the blood may function in greatly reducing loss by diffusion.

Since the curly top virus is able to retain its activity for 4 weeks or more in certain extracts from plant juice and for 10 months (*2*) or

more in dried phloem content, it seems probable that it would retain its activity for relatively long periods in a medium as favorable as the blood is presumed to be. Once the virus enters the blood, loss would be restricted to that resulting from inactivation and from diffusion into the salivary glands and possibly back into the alimentary tract. Assuming no multiplication of virus in the insect, the time that a leafhopper would remain viruliferous when deprived of new sources of virus would depend, therefore, on the original amount of virus carried, on rate of inactivation, and on rate of loss by diffusion into the salivary glands and into the alimentary tract. If the initial virus charge was high and losses were small, the leafhopper would retain its ability to transmit virus over a relatively long period.

Of the factors influencing retention of virus by the leafhopper, only size of initial charge can be governed experimentally. Decreasing the size of the initial virus charge definitely shortened the time in which the leafhoppers were able to produce a maximum amount of infection. This, together with the evidence that the virus content decreased with time regardless of the size of the initial charge, indicates lack of reproduction of the virus in the leafhopper. If the same condition holds generally among insect vectors that retain viruses for long periods, much of the evidence now accepted as supporting the idea that certain plant viruses multiply in their insect vectors must be reevaluated.

The virus evidently passes from the blood into the salivary glands, where it is mixed with the salivary secretions and passed into the plant when the leafhoppers feed. The relatively small amount of virus obtained from salivary glands removed from the leafhopper shows that these glands are not the chief virus reservoir. Other evidence suggests that the salivary glands may be somewhat unfavorable to the virus. It may be that the failure of leafhoppers to produce a normal amount of infection in the first and second 6-minute feedings after a long fasting period is due to partial inactivation of virus in the salivary glands by long contact with salivary secretions.

A more thorough study of the relation of the curly top virus to different tissues and fluids of nonvector species of insects, such as *Aceratagallia californica* and *Myzus persicae*, that pick up large quantities of virus and retain it for considerable periods, should shed light on the question of insect specificity in virus transmission. Since the virus retained its activity for several days in the bodies of the two species of insects named above and since both fed in the phloem, their inability to cause infection was not due to lack of active virus or to the tissue on which they fed, but evidently resulted from the presence of an effective barrier to virus passage in some part of the insect.

SUMMARY AND CONCLUSIONS

Beet leafhoppers, *Eutettix tenellus* (Baker), picked up virus from diseased beet plants in a feeding time of 1 minute, and viruliferous leafhoppers infected healthy plants in a feeding time of 1 minute. However, the minimum time for an individual leafhopper to pick up virus from a diseased plant and transmit it to a healthy plant was 4 hours.

Nonviruliferous leafhoppers placed on diseased plants acquired sufficient virus in 2 days to give them their maximum ability to infect

seedling beets. A longer feeding period was necessary to enable them to acquire their maximum charge of virus.

A fasting period of 18 hours decreased the ability of leafhoppers to produce infection in the first two 6-minute feedings following the fast period. Feedings following a fast period of 15 minutes to 3 hours produced more infection than those following fast periods of 1 to 6 minutes or those following fast periods longer than 3 hours.

Virus was obtained from the blood, salivary glands, feces, and alimentary tract of the leafhopper, indicating a rather general distribution of the virus in the insect. However, the more abundant recovery of virus from the blood indicates that the blood is the chief virus reservoir.

Both virus content and ability to cause infection gradually decreased over periods of 8 to 10 weeks in viruliferous leafhoppers when the leafhoppers were confined to a very resistant or immune plant (Australian saltbush or sweet corn). This was true regardless of the size of the initial charge of virus in the leafhoppers when placed on the resistant or immune plant.

Leafhoppers that had a low charge of virus, acquired by a 6-hour feeding on an extract from viruliferous leafhoppers, lost the power to cause infection after 54 days when transferred daily on small beet plants. However, leafhoppers that had lost the power to cause infection regained it when allowed a short feeding period on curly top beets. These facts prove conclusively that if there is any multiplication of the virus in the leafhopper, it is not sufficient to maintain the original virus content. It seems probable that there is no multiplication of virus in the leafhopper.

Individual leafhoppers vary greatly in their ability to transmit virus. The results of selecting and mating leafhoppers in successive generations indicated that it is possible to produce strains lower than normal and higher than normal in ability to transmit virus to seedling beets in short-interval feedings. However, no individuals inherently lacking in ability to transmit virus were discovered and the results indicate that if such individuals exist within the species they are rare.

The following nonvector species of insects acquired virus by feeding on curly top plants: *Myzus persicae, Aphis rumicis, Pemphigus betae, Aceratagallia californica, Phlepsius strobi, Empoasca solana, Hercothrips femoralis,* and *Tetranychus* sp. When transferred to healthy plants, these insects retained the virus for periods ranging from less than 1 day, in the case of *H. femoralis,* to 21 days, in the case of *A. californica.*

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INFLUENCE OF CERTAIN HARMFUL SOIL CONSTITUENTS ON SEVERITY OF PYTHIUM ROOT ROT OF SUGARCANE [1]

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INTRODUCTION

The severity of root rot of the very susceptible noble varieties of sugarcane (*Saccharum officinarum* L.), and particularly of the more tolerant and now generally planted hybrid seedlings in Louisiana, depends partly at least upon the intensity of various host-conditioning factors. The principal root-rotting fungus, *Pythium arrhenomanes* Drechsler, may be readily isolated from practically every field and from the root systems of both good and poor cane. Its dispersion through the soil is favored by the usually abundant rainfall apparently whenever conditions, of either transitory or permanent nature, permit abundant root infections and rapid decay.

A high water table combined with fine texture of soil and low winter and spring temperatures are probably the chief indirect causes of severe outbreaks of root rot on present commercial varieties. Poor drainage in the Louisiana sugar belt is characteristic of the extensive low-lying areas of Sharkey clay and many of the transitional "mixed lands" separating the former from the higher lying sandy loams bordering the Mississippi River and numerous bayous. These heavy clays and clay loams have been variously estimated to occupy from 20 to 40 percent of the average sugarcane farm. With frequent and excessive rainfall they may remain practically saturated for periods of a week or longer. This would favor the possible formation of substances in the soil inimical to cane growth. Some reduction of both inorganic and organic soil compounds might be expected, with at least the formation of subtoxic concentrations of some of the reduced products. Should these exert a predisposing effect on the roots for pythium attack, the unusually severe root rot, arrested growth, yellowing of foliage during periods of drought, and delayed stooling suffered during some seasons by ordinarily root rot-tolerant varieties might be explained.

During the course of a detailed investigation of the relation of environmental factors to the severity of root rot the possible predisposing effect of various deleterious substances that may arise under conditions of poor soil aeration and drainage was studied. This paper presents the results of such experiments with hydrogen sulphide and salicylic aldehyde.

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[2] The writers are indebted to P. R. Dawson, senior biochemist, Division of Soil Fertility Investigations, Bureau of Plant Industry, for critical reading of the manuscript.

REVIEW OF LITERATURE

No soils investigations have been conducted in relation to this specific problem in Louisiana, although Flor (*1*) [3] attempted to determine whether the severe root rot in seepage areas along the Mississippi River might not be due to the accumulation of toxins. His failure to demonstrate toxins might possibly have been due to oxidative and other beneficial changes taking place in the untreated seepage soil during the considerable period between its collection and use in his greenhouse experiments.

In studies of the decomposition of cane trash in Louisiana sugarcane soils, Sturgis (*13*) found that intermittent high moisture during late winter and spring greatly retarded decomposition and nitrification and appeared to bring on a toxic condition which disappeared relatively slowly when better drainage and higher temperature prevailed. More recently Sturgis (*14*) has reported on the effect of waterlogging and organic matter on the rate, intensity, and amount of reduction in Sharkey soils incident to the growing of rice. Addition of organic matter markedly increased the intensity of reduction and accumulation of reduced iron and manganese. Robinson (*4*) found unusually large amounts of soluble iron and manganese and the presence of sulphides in poorly drained spots in fields where corn grew poorly.

With respect to the occurrence of harmful organic compounds in soils, the earlier extensive investigations of Schreiner, Shorey, Skinner, and associates (*5*, *6*, *7*, *8*, *9*, *10*, *11*) may be cited. They isolated a whole series of injurious compounds from unproductive soils characterized by poor aeration, low biologic activity, deficient or poor soil preparation, and excessive moisture content. Deficiency of oxygen in the decomposition of plant residues caused the accumulation of such intermediate and injurious products as vanillin, salicylic aldehyde, acrylic acid, dihydroxystearic acid, and benzoic acid, all of which were injurious to plant growth in relatively low concentrations in water cultures.

MATERIALS AND METHODS

A standardized method for growing sugarcane in sand-nutrient cultures and the writers' root rot inoculation procedure have been partly described in an earlier publication (*2*). The method consists essentially in the use of a high-purity Pennsylvania quartz sand in 3-gallon glazed crocks provided with a ½-inch drain hole in the bottom from which a rubber tube conducts excess liquid to a 2-quart mason jar placed below each crock (fig. 1). The jar is painted black to prevent the growth of algae. It holds approximately 1,900 cc, and the sand in the crock, depending upon its fineness, retains against gravity about 2,000 to 2,600 cc, making a total of about 4 to 4.5 liters in the system. Daily, or twice daily, between changes of nutrient, the contents of the jar are poured over the sand in the crock, and any deficiency of leachings noticeable in the jar from evaporation and transpiration during the preceding day is made up by the addition of distilled water. This procedure not only simulates the probable effect of rain in dispersal of and infection by the fungus, but tends to equalize concentration of the solution throughout the culture.

[3] Reference is made by number (italic) to Literature Cited, p. 67.

The nutrient solution is partly or completely renewed at weekly or longer intervals, according to the requirements of the plants. Hydrogen-ion determinations on fresh leachings are made weekly or more frequently, and the ratio of ammonia to nitrate ions is so varied as to maintain a slightly acid reaction, about pH 6.0, which is favorable



FIGURE 1.—Method of growing sugarcane in sand-nutrient cultures. A rubber drain tube from the bottom of each crock conducts excess nutrient into the jars, the contents of which are periodically made up to volume and poured back into the crocks to equalize the concentration throughout the culture.

for cane growth. The nutrient solution D, used in most of the writers' experiments, contains the following salts in the partial volume-molecular concentrations indicated: NH_4NO_3, 0.00225; $Ca(NO_3)_24H_2O$, 0.00225; KH_2PO_4, 0.0015; K_2HPO_4, 0.0005; $MgSO_4$, 0.001; KCl, 0.0025; plus rarer elements in parts per million, Fe 5.0, Mn 1.0, B 0.5, Al 0.5, Cu 0.25, Zn 0.25, Br 0.25, and traces of Si and Ti.

Young sugarcane plants of uniform height and stature, previously sprouted in 4-inch pots of sand, are reset in the crocks with their attached 2- to 3-inch cutting and without disturbing the roots. After a week to 10 days the plants have become established and are supported at least partly by the development of their permanent or shoot root system. At this time treatments are applied and the various replicates of the treatments are placed in randomized arrangement on the greenhouse benches. After another week the cultures that are to receive the root-rotting pythium are inoculated.

Since an important effect of root rot is a retardation of growth and reduction in the number of suckers, or tillers, each experiment was continued for 2 to 3 months, or until further growth represented merely elongation of existing shoots. This was followed by weekly measurements of height of all shoots per crock. Harvesting after 2 to 3 months gave practically the same percentage difference between objects as after 7 months or until nearly full-grown stalks had developed.

HYDROGEN SULPHIDE

Hydrogen sulphide at initial concentrations of 10 and 50 p. p. m. in nutrient D was studied for effect on growth and root rot of the very susceptible D–74 variety grown in quartz sand cultures. The cuttings were sprouted and a uniform group of plants established in 3-gallon crocks, with two plants per crock. The hydrogen sulphide gas produced from iron sulphide in a Kipp generator was first bubbled through a water trap to remove any accompanying hydrochloric acid and then conducted into a measured volume of distilled water until the water was saturated. The temperature of the saturated solution and published solubility data permitted calculation of the volume of solution required to be added to each lot of dilute nutrient to give hydrogen sulphide concentrations of 10 and 50 p. p. m., respectively. The rarer elements ordinarily added to the nutrient were withheld because the iron, copper, and zinc salts would be precipitated by the hydrogen sulphide. They were added the following day to the receiving jars before the contents of the jars were poured over the crocks.

Inoculations were made May 5, 1932, with the authors' mildly parasitic strain No. 730 of *Pythium arrhenomanes*. The treatments were started a week later. Each treated series was divided into six inoculated and six control crocks, and a similar untreated series served for comparison. Fresh treatments were applied weekly. Under the well-aerated condition of these cultures, the hydrogen sulphide was evidently rapidly oxidized, since no odor was detectable in displaced nutrient that had occupied the sand for 24 hours. This was accompanied by a slightly greater increase of acidity in the nutrient of the 50-p. p. m. treatment.

Twice weekly and, for a period, daily determinations of pH revealed a rapid increase in acidity in all series from the initial reaction of pH 6.0 for fresh nutrient to as high as pH 3.8 and 4.0 at the end of the first month. This was due to the use of ammonium nitrate as the sole source of nitrogen in nutrient D. Thereafter part of the total nitrogen was added as calcium nitrate, and the ratio of the two salts was varied to maintain the reaction between pH 5.2 and 6.5.

The experiment extended from May 5 to July 26, 1932. The plants in the untreated and uninoculated series particularly showed excellent development, and those in the remaining series, while much retarded and deficient in suckering, showed at no time any unhealthy appearance of foliage. Mean total height of plants and dry weight of tops per crock are given in table 1.

TABLE 1.—*Effect of hydrogen sulphide on growth and root rot of the very susceptible D–74 variety of sugarcane*

Treatment	Total length of shoots			Dry weight of tops		
	Mean per crock	Reduction due to—		Mean per crock [1]	Reduction due to—	
		H_2S	Fungus		H_2S	Fungus
	Centimeters	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
None:						
Controls	158			95.9		
Inoculated	86		46	66.3		30.9
H_2S, 10 p. p. m.:						
Controls	128	19		75.6	21.2	
Inoculated	79		38	52.6		30.4
H_2S, 50 p. p. m.:						
Controls	125	21		70.7	26.3	
Inoculated	70		44	45.2		36.1

[1] Difference between means required for odds of 19 : 1=9.8 g, and for odds of 99 : 1, 13.1 g.

Mean plant weights and calculations in table 1 indicate severe damage from the sulphide treatments in the absence of the fungus. This was expected in view of the well-known poisonous action of soluble sulphides on most crops. The noticeably darkened appearance of the sand receiving the 50 p. p. m. of hydrogen sulphide indicated extensive precipitation of insoluble sulphides on the particles, but the roots remained white and were merely deficient in length and branching.

Any weakening or unhealthy effect of the hydrogen sulphide apparently did not cause significantly greater damage by the fungus, as the last column of table 1 shows. In fact, the yield reductions caused by the fungus are not significantly different in any of the series, which suggests that in the concentrations employed hydrogen sulphide was without influence on susceptibility of the plants to root rot.

SALICYLIC ALDEHYDE

Among the many harmful organic compounds isolated by Schreiner, Shorey, Skinner, and associates from unproductive soils were several of aldehyde nature (*8*) that proved to be common constituents of the soil organic matter. Salicylic aldehyde, vanillin, and trithiobenzaldehyde were definitely identified and studied in relation to crop growth. They were considered to have been either directly introduced in certain plant remains or formed as intermediate reduction products during the decomposition of vegetable residues under conditions of high acidity or in soils low in oxidizing power and biological activity.

Salicylic aldehyde was identified by Shorey (*10*), and greenhouse and field tests showing its injurious effect on plant growth were reported by Schreiner and Skinner (*8*) in 1914. More comprehensive

studies on the occurrence and persistence of aldehydes in garden and field soils and their influence on crop production were summarized by Skinner (*11*) in 1918. Ten parts per million of salicylic aldehyde in nutrient solution cultures reduced the growth of wheat by 27 percent. Generally, less damage resulted from additions to soils; in fact, no detrimental effect whatever was determined for certain fertile soils well supplied with lime and phosphates.

Since the occurrence and persistence of aldehydes in soils were often associated with poor drainage and low or temporarily interrupted biological activity—conditions which characterize large areas in late winter and spring in the Louisiana sugar district—it seemed advisable to determine the possible influence of aldehydes on growth and root rot of sugarcane. However, as previously mentioned, it has not been demonstrated that such substances actually occur in Louisiana soils.

Preliminary greenhouse tests with commercial preparations of both salicylic aldehyde and vanillin suggested greater tolerance of sugarcane to these compounds than Schreiner and Skinner observed in several other crops. However, these differences may be attributable to differences in the nutrient solutions and technique employed. Therefore, a commercial preparation of salicylic aldehyde, selected for ease of handling, was tested in a series of more adequately replicated greenhouse experiments.

First, it was necessary to ascertain what effect this compound would have on the root-rotting pythium alone. Some idea of this was gained by comparing growth of the fungus on agar containing different concentrations of the chemical. Concentrations of 10, 25, and 50 p. p. m. were made up in melted and previously sterilized corn-meal agar in flasks, and a series of Petri dishes was poured with each, besides which there was an untreated series. On cooling, the dishes were inoculated at one side with a pure culture of *Pythium arrhenomanes*. Average rate of growth after 24 hours revealed no significant differences between the control and the 10 and 25 p. p. m. series; in the 50 p. p. m. no growth whatever had taken place, and subsequent examination showed that the fungus had been killed in all plates of this series. In the lower concentrations, therefore, salicylic aldehyde was without visible effect on the appearance and rate of growth of the pythium in culture.

Next, preliminary qualitative tests were conducted to determine the persistence of salicylic aldehyde in 3-gallon crocks of sand without plants but provided with drain tubes and receiving jars as in the experiments. Most of the liquid in the sand of two crocks was replaced by additions of nutrient containing 25 and 50 p. p. m., respectively, of the aldehyde. At daily intervals the solution was displaced into the receiving jars and aliquots were removed and tested against standards with ferric chloride. The contents of the receiving jars that contained the aldehyde were then poured back over the sand and similarly replaced and tested on succeeding days.

Seventy-two hours after the original application there was no longer any visible reaction for aldehydes with ferric chloride in the case of the 25 p. p. m. dosage, and only a faint change in color was noted in the leachings of the 50 p. p. m. application. The latter was similar to that of a 5 p. p. m. control, which concentration is about the minimum detectable by the naked eye with this indicator. Therefore, within

3 days most of the salicylic aldehyde applied to this sand was apparently absorbed or oxidized.

EXPERIMENT 1

In experiment 1 an approximate concentration of 20 p. p. m. salicylic aldehyde in nutrient solution was tested in sand cultures of sugarcane in the presence and in the absence of the moderately virulent strain 58 of *Pythium arrhenomanes*. Comparable control series with nutrient alone with and without the fungus served for comparison of separate and combined effects of treatment and pythium.

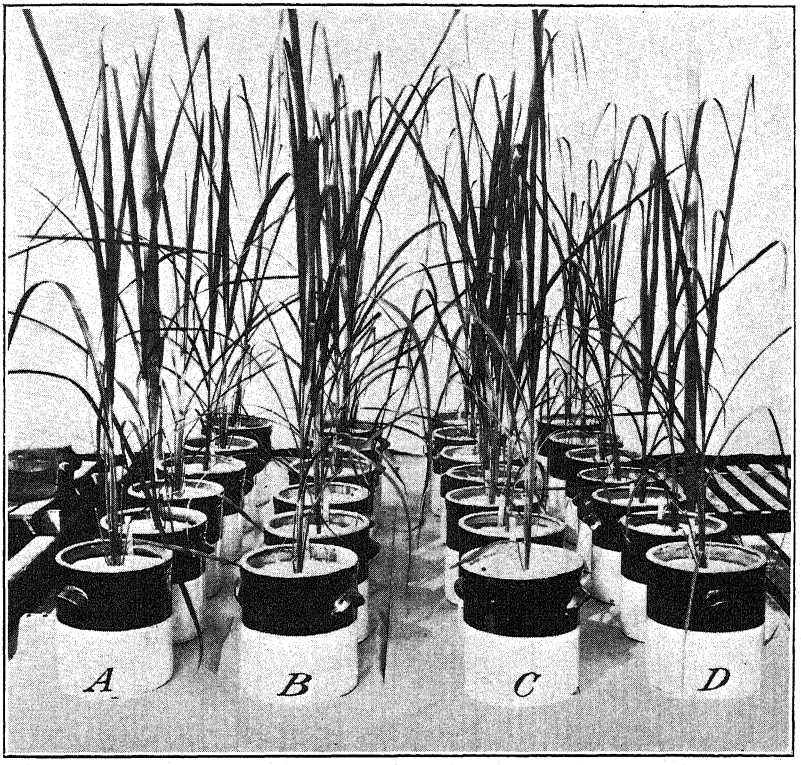


FIGURE 2.—Influence of salicylic aldehyde on susceptibility of sugarcane to root rot in sand-nutrient cultures: *A*, Control; *B*, aldehyde alone; *C*, pythium alone; *D*, combination of aldehyde and pythium.

Thirty-three glazed crocks of 3-gallon capacity were filled with new quartz sand, wetted with tap water, autoclaved for 2 hours at 15 pounds' steam pressure, cooled, and leached with 2 liters of distilled water each. The crocks were provided with a central drain hole in the bottom, but in this test neither drain tubes nor receiving jars were employed. On December 8, 1931, a uniform series of young plants of the moderately susceptible Co. 281 variety growing in 4-inch pots of similarly steamed sand were transplanted, one per crock, and given 1 liter of distilled water. On December 10, inoculations with strain 58 of *Pythium arrhenomanes* were made to the fungus series and similar but sterile media were added to the controls. On December 14, 1

liter of nutrient solution containing 50 p. p. m. salicylic aldehyde was added to the aldehyde series, and nutrient alone to the controls. Uncertain, and at the time inadequate, supplies of distilled water prohibited the addition of exactly 20 p. p. m. of the aldehyde in a volume of nutrient sufficient to replace at one time all liquid (approximately

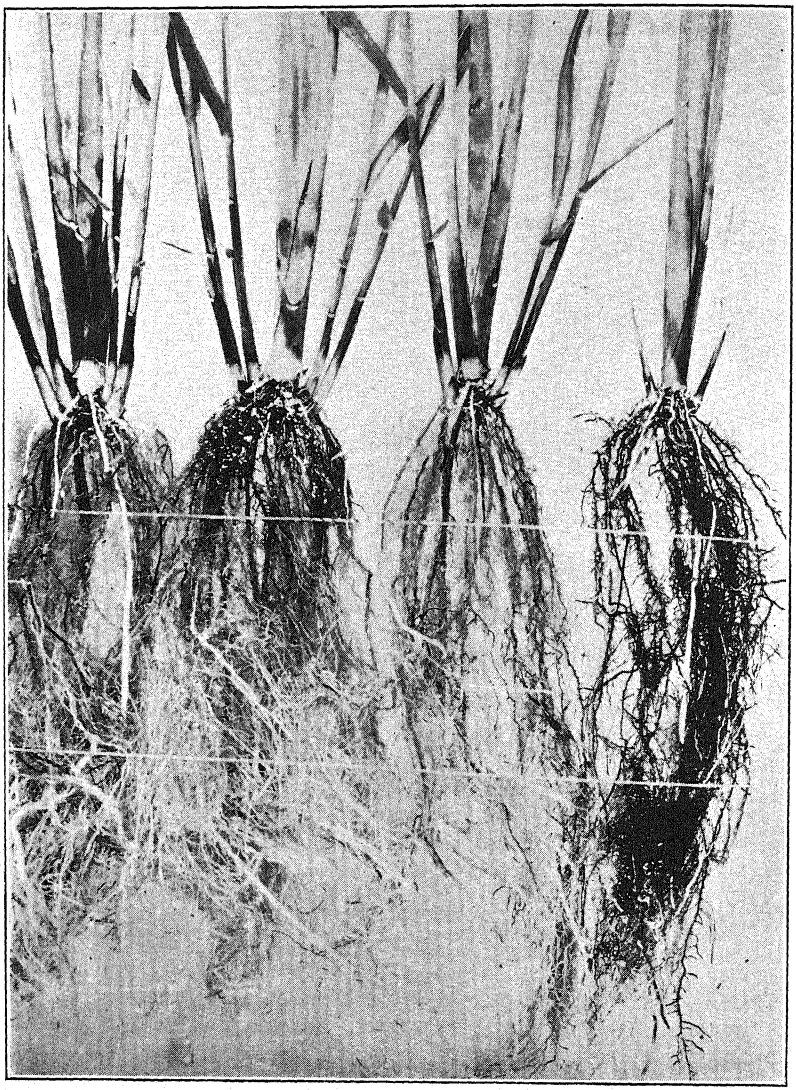


FIGURE 3.—Comparative extent of suckering (tillering) and condition of root systems of representative plants in experiment 1. Left to right: Control, aldehyde alone, pythium alone, and aldehyde plus pythium.

2,500 cc) retained against gravity by this sand. However, the 50 p. p. m. salicylic aldehyde in 1,000 cc of nutrient actually applied is assumed to have diffused to some extent through the approximately 1,500 cc of liquid not displaced from the sand and was thereby diluted to about 20 p. p. m. of total liquid. Tests indicated that some diffusion

at least had taken place soon after application, because the addition of another liter of nutrient or distilled water gave leachings with sufficient aldehyde for detection with ferric chloride. Furthermore, growth measurements of the plants showed that the fungus was active, whereas in the aforementioned test in agar cultures it had been killed in a concentration of 50 p. p. m. Nevertheless, it was unfortunate that a more uniform initial concentration of the chemical could not be placed in contact with the root systems.

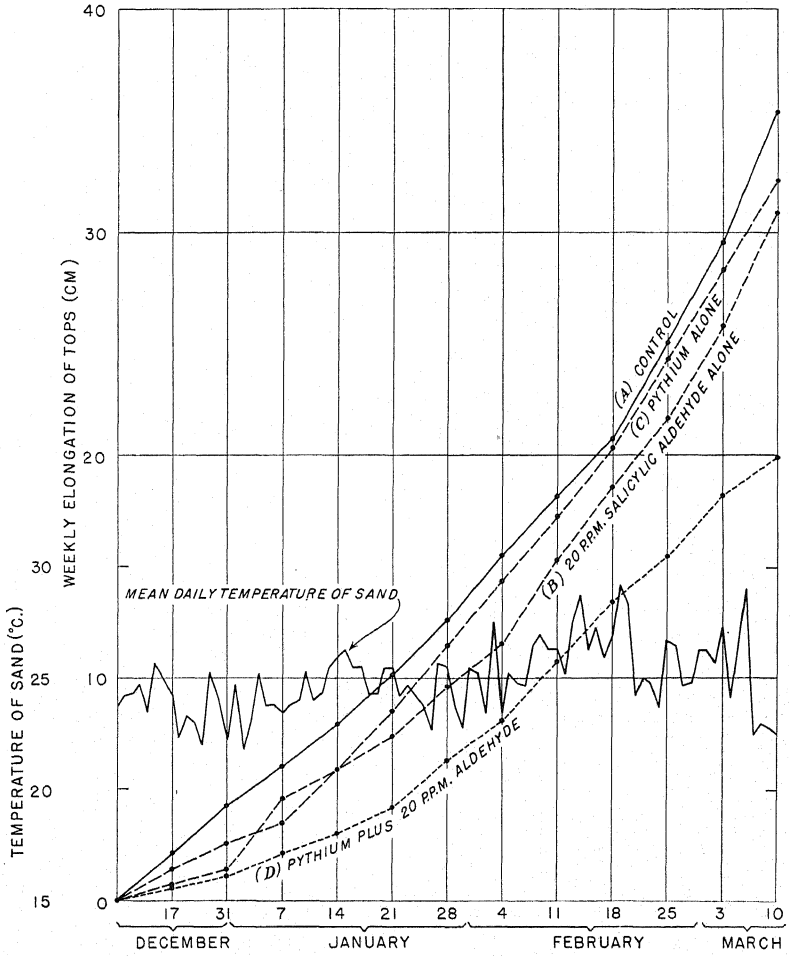


FIGURE 4.—Mean daily temperature of sand, and weekly elongation of tops *A–D*, reflecting damage from root rot in the presence and in the absence of salicylic aldehyde in sand cultures.

The experiment extended from December 10, 1931, to March 10, 1932. Growth was normal in appearance and extent for this season of the year, with gradual acceleration in rate of elongation as temperatures rose and the period of sunshine increased toward the end of the test. Plants receiving both the treatment and fungus (series D) often showed temporary wilting at midday, which indicated a deficiency of absorbing root area. Figure 2, from a photograph taken

nearly 3 weeks prior to the termination of the experiment, illustrates the smaller size in series D and the absence of noticeable differences between the remaining series.

Striking damage from the combination of aldehyde and fungus was revealed on examination of the root systems, as is shown by the representative comparison in figure 3. Whereas the fungus alone caused extensive rootlet tip damage, in the presence of the aldehyde it rotted completely most of the finer roots and invaded and suppressed many of the larger ones. The greatly reduced absorbing root area that resulted was reflected in a slow elongation of the primary shoot and an absence of normal suckering (tillering).

Mean height and green weight of plants are presented in table 2 and shown graphically in figure 4. There was no significant difference between the controls and the aldehyde-treated plants in the absence of the fungus. The fungus alone caused the usual significant reduction for greenhouse tests with this variety, but in the presence of the aldehyde the damage was more than twice as great, which suggests a predisposing effect of the aldehyde for root rot.

TABLE 2.—*Effect of salicylic aldehyde on growth of sugarcane variety Co. 281 and the severity of root rot caused by Pythium arrhenomanes in experiment 1*

Series	Treatment	Plants	Mean height	Difference due to—		Mean green weight of tops [1]	Difference due to—	
				Aldehyde	Pythium		Aldehyde	Pythium
		Number	*Centimeters*	*Per cent*	*Per cent*	*Grams*	*Per cent*	*Per cent*
A	None (controls)	6	35			180.3		
B	Aldehyde alone, 20 p. p. m.	7	31	−11		185.1	+2.7	
C	Pythium alone	10	32		−8	141.3		−21.6
D	Pythium plus 20 p. p. m. aldehyde	10	20		−35	90.3		−51.2

[1] Difference between means required for odds of 19 : 1=26.7 g., or 14.8 percent of control (A) series.

EXPERIMENT 2

Experiment 1 was repeated with the following modifications as experiment 2: Weekly applications of 50 p. p. m. of salicylic aldehyde on the basis of 2 liters of nutrient D were made to sand cultures of Louisiana Purple, a very susceptible variety, and to C. P. 807, a variety highly resistant to root rot, both in the presence and in the absence of the mildly parasitic strain No. 730 of *Pythium arrhenomanes*. Large galvanized cans, 14 inches in diameter by 24 inches high, coated inside with paraffin and holding 100 pounds of sand, were used in place of crocks. The cans were provided with drain outlets and receiving jars. Four plants were set in each can, and there were five cans per object, two with Louisiana Purple and three with C. P. 807.

The test plants were set in the cans on March 24 and inoculated March 25, and the first aldehyde application was made on March 28, 1932. Five additional applications in 2 liters of nutrient were made at weekly intervals until May 10, when the plants were harvested. Weekly pH determinations on leachings gave values between pH 5.4 and 6.2. Daily the receiving jars were poured over the sand and

sufficient distilled water was added to maintain a fairly uniform moisture content. This procedure provided a 24-hour exposure of most of the roots to each full-strength (50 p. p. m.) aldehyde application once a week, which was followed by rapid diminution in concentration of the aldehyde until the next application.

All plants of both varieties made normal appearing growth and, except for the Louisiana Purple that received the combination of aldehyde and pythium, the plants tillered profusely. The average green weights per individual plant, shown in table 3, reveal for the susceptible Louisiana Purple variety a significantly predisposing effect from the aldehyde similar to that obtained in the preceding experiment (table 2). However, the root-rot-resistant C. P. 807 variety shows no significant reduction from either the combination or the pythium alone.

TABLE 3.—*Influence of salicylic aldehyde on growth and severity of root rot of susceptible and resistant varieties of sugarcane, experiment 2*

Variety and treatment	Height of primary shoots	Difference due to—		Mean green weight of tops [1]	Difference due to—	
		Aldehyde	Pythium		Aldehyde	Pythium
	Centimeters	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
Louisiana Purple (susceptible to root rot):						
Untreated controls	21			166.1		
Aldehyde alone, 50 p. p. m	18	−14		144.9	−12.8	
Pythium alone	16		−24	141.7		−14.7
Pythium plus 50 p. p. m. aldehyde	14		−22	99.6		−31.3
C. P. 807 (resistant to root rot):						
Untreated controls	51			183.3		
Aldehyde alone, 50 p. p. m	40	−22		143.8	−21.5	
Pythium alone	48		−6	165.2		−9.9
Pythium plus 50 p. p. m. aldehyde	40		0	137.0		−4.7

[1] Means of individual plants with suckers; differences required for odds of 19:1 are, for Louisiana Purple, 28.7 g, or 17.3 percent of controls; and for C. P. 807, 33.7 g, or 18.4 percent of controls.

EXPERIMENT 3

Since an adequate supply of moisture was difficult to maintain in the upper portion of the deep cans employed in the preceding test, experiment 1 was repeated to test further the apparent predisposing effect of salicylic aldehyde on susceptibility to root rot. Six crocks with two plants each were used for comparing two concentrations of the aldehyde in the presence and in the absence of the fungus. The variety Co. 281 and the same strain (No. 58) of the fungus were again employed, but drain tubes and receiving jars were used to maintain a more uniform concentration of nutrient solution throughout the sand. Nutrient solution D was applied 2 liters per crock every other week, which provided ample nutrition for the plants.

Salicylic aldehyde was added to the 2 liters of nutrient solution at each biweekly partial renewal to make initial concentrations of 40 and 80 p. p. m., respectively. These were subsequently reduced by diffusion and some percolation into the receiving jars. Daily the contents of the jars were poured over the sand after being brought up to capacity with distilled water. Since they also held approximately 2 liters, the result was substantially an alternate day exposure of the roots to the aldehyde until it became oxidized or adsorbed by the

sand, i. e., after 4 to 6 days. Applications of nutrient and aldehyde were made on September 12, 4 days after planting, and repeated September 26, October 10 and 24, November 7 and 21, and December

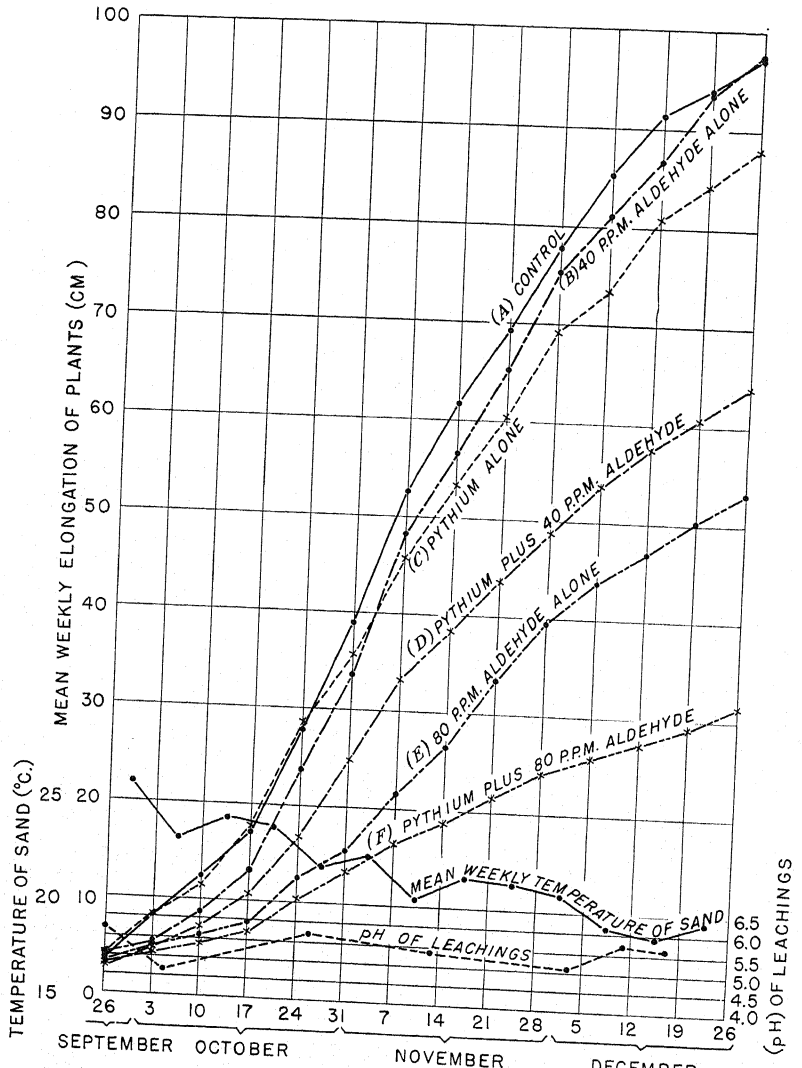


FIGURE 5.—Average weekly temperature of sand, pH of leachings, and growth curves *A–F* of plants grown in two concentrations of salicylic aldehyde in the presence and in the absence of the root-rotting fungus *Pythium arrhenomanes*.

5, 1934, making a total of seven partial renewals. Inoculations were made on September 14, and the test plants were harvested on December 28, 1934. The results are presented in table 4 and figure 5.

TABLE 4.—*Effect of biweekly applications of salicylic aldehyde on growth and severity of root rot of the moderately susceptible sugarcane variety Co. 281, experiment 3*

Series	Treatment (6 replications)	Mean total height per crock	Difference due to—		Mean green weight of tops [1]	Reduction due to—	
			Aldehyde	Pythium		Aldehyde	Pythium
		Centimeters	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
A	None (controls)	195			588.4		
B	Aldehyde alone, 40 p. p. m.	196	+0.5		564.4	4.1	
C	Pythium alone	177		−9	556.0		5.5
D	Pythium plus 40 p. p. m. aldehyde	128		−35	389.2		31.0
E	Aldehyde alone, 80 p. p. m.	107	−45		323.2	45.4	
F	Pythium plus 80 p. p. m. aldehyde	63		−41	204.6		36.7

[1] Differences between means required for odds of 19 : 1=83.6 g, and for 99 : 1=113.8 g.

The results of this experiment confirm those of experiment 1 (table 2) in demonstrating a significantly aggravating effect of salicylic aldehyde on root rot. Whereas alone neither the lesser concentration of the aldehyde nor the fungus caused appreciable damage, when the two were combined the plants were severely stunted. The growth curves in figure 5 suggest that the pythium was also more active at higher temperatures in the presence of the aldeyhde than in its absence. When the aldehyde was absent there was pronounced retardation of growth at temperatures below that required for vigorous cane growth (about 20° C.).

Applications of 80 p. p. m. of the aldehyde caused severe growth retardation without the aid of the fungus. Preliminary tests had shown that weekly dosages of 100 p. p. m. would cause severe wilting and eventual death of the plants, but the less frequent applications of 80 p. p. m. in this test resulted in no such abnormal external symptoms in the absence of the fungus. The roots were merely deficient in length and extent of branching and to all outward appearances were not different from untreated plants of the same size but much younger age. The pythium, no doubt, was also affected by the strong concentration of aldehyde, although with the gradual weakening or disappearance of the latter during the long intervals between successive applications, it was apparently still able to cause about seven times as much growth reduction as it had in the absence of aldehyde.

DISCUSSION

The significant increase in severity of root rot of sugarcane in the presence of salicylic aldehyde in these experiments is interpreted as the result of a conditioning or predisposing effect of the chemical on the roots which enabled the fungus to cause greater damage. The possibility that it might be due to a stimulating or nutritional effect on the fungus would appear to be excluded by the negative results from tests of the fungus in pure culture.

The results of these experiments suggest the desirability of soil investigations to explain certain anomalies connected with the occurrence of the disease in the field. Should this or similarly behaving

compounds be found in the root rot soils, possible corrective measures, such as improved drainage, special fertilization, and soil amendments, could be more intelligently instituted. The effect of increasing biological activity, improving aeration, moisture control, and possibly chemical conditions of such soils, on root health as well as on possible formation and accumulation of injurious or root rot-accentuating substances should be determined. The results of experiments by Robbins and Massey (*3*) suggested that the aldehyde vanillin is destroyed in the soil by certain micro-organisms, and Skinner and Noll (*12*) showed that the injurious effect on plants from its persistence in an unproductive, acid soil could be overcome by the addition of lime or of lime and phosphate. Possible insufficiency of these materials is not believed to have been a factor in the tests with salicylic aldehyde reported in this paper, because of the excellent growth of treated, uninoculated plants. Each 2-liter weekly or biweekly addition of nutrient solution D provided the equivalent of 25.9 pounds of calcium oxide and 29.3 pounds of phosphorus pentoxide per acre.

The cultivation of vigorous, root-rot-resisting varieties of sugarcane, such as Co. 290, C. P. 28/11, and C. P. 29/116, on the poorly drained, heavy clay soils of the Louisiana sugar district has assured, for the present at least, economical utilization of such areas. However, the question naturally arises: Should varieties alone without accompanying soil improvement be depended upon as a permanent or even as the most economical solution of production problems on such soils? A tentative answer is found in the renewed efforts of many growers to improve drainage by deepening field ditches and outlet canals and also to prevent caking, and at the same time to raise the general level of fertility by plowing under legumes and cane trash and occasionally by applications of factory filter-press cake and barnyard manure. Under such treatment the root-rot-resistant varieties have shown a marked response, and in a few instances the improvement has been so great that excellent yields of the rather susceptible Co. 281 have been obtained.

SUMMARY

During an investigation of host-conditioning factors that influence the severity of root rot caused by *Pythium arrhenomanes*, in the poorly drained, heavy clay soils of the Louisiana sugar district, the possible predisposing effect of deleterious substances, which might arise during temporary water-logging, was determined. Hydrogen sulphide and salicylic aldehyde were selected as examples and tested in greenhouse inoculation experiments with sugarcane growing in sand-nutrient cultures.

Hydrogen sulphide alone at concentrations of 10 and 50 p. p. m. in nutrient solution reduced the growth of tops approximately 21 and 26 percent, respectively; the pythium alone, 31 percent, and in combination with the sulphide, 30 and 36 percent, which latter percentages are not sufficiently different to indicate any special influence of the sulphide on disease intensity.

Salicylic aldehyde significantly increased susceptibility to root rot. Concentrations of 20 to 40 p. p. m., which had little if any influence on cane growth in the absence of the fungus and showed no effect on

the fungus in culture, apparently so predisposed the roots to infection that when the fungus was present the reduction in weight of plants was from two to seven times greater than from the fungus alone.

The presence of this or similarly behaving compounds in the poorly drained, heavy clay soils of the Louisiana sugar district would account at least partly for the frequently severe root rot noted in all but the most resistant varieties. Improving the drainage and the general level of fertility in such areas has in numerous instances markedly increased yields of even the resistant varieties.

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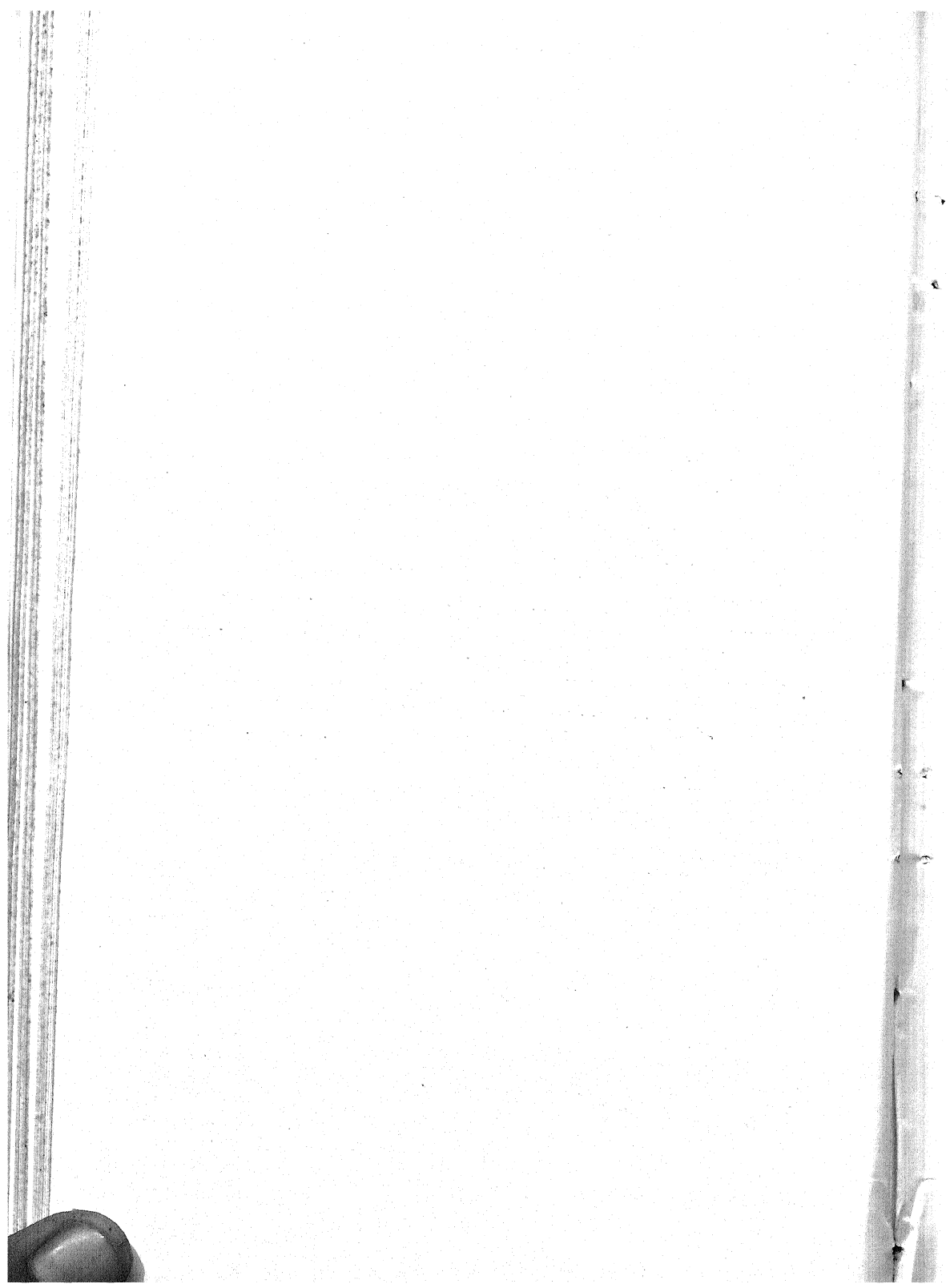
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THE VITAMIN A VALUE OF BLUE GRAMA RANGE GRASS AT DIFFERENT STAGES OF GROWTH[1]

By MARGARET CAMMACK SMITH, *nutrition chemist, Department of Nutrition,* and E. B. STANLEY, *head, Department of Animal Husbandry, Arizona Agricultural Experiment Station*

INTRODUCTION

Livestock in the range area of Arizona, depending as they do almost exclusively upon native vegetation for their feed supply, are obviously subjected to conditions which make for a widely variable supply of the several feed essentials. Although Woods and her coworkers[2] have reported many range forages to be excellent sources of vitamin A, Hart and Guilbert[3] found that vitamin A deficiency occurred in range cattle under natural conditions, the deficiency developing when the dry feed was unusually prolonged.

In Arizona the grama grasses are the most widely distributed and the most valuable of the perennial grasses used for grazing purposes. Blue grama (*Bouteloua gracilis*) is probably the most abundant of these grasses between the elevations of 4,000 and 8,000 feet and is particularly prevalent on the grassland ranges. It grows to a height of from 10 to 20 inches and develops a good growth of fine basal leaves which remain partly green during the winter months, the extent of greenness depending upon the moisture available. The annual growth of this important range forage is initiated by the summer rains in late July. The plant grows rapidly, matures quickly, and by the middle of October begins to dry up. Except for some greening of the basal leaves in the early spring it shows no further growth until the following summer rains. During the dormant stage, the remaining old-growth part of the plant is dry and comparatively unpalatable.

As blue grama grass constitutes one of the principal sources of vitamin A for range cattle in certain sections of Arizona, the authors have sought to ascertain the vitamin A potency of this nutrient for range cattle at different stages of its growth.

EXPERIMENTAL PROCEDURE

The samples of blue grama used in this study were collected from a grassland type of range in southern Arizona in the vicinity of Sonoita at an elevation of 4,000 feet. Three sets of samples were taken at different stages in the growth of the plant. The first collection was made the first week in August 1936, about 2 weeks after

[1] Received for publication July 19, 1937; issued March 1938.

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[3] HART, G. H., and GUILBERT, H. R. VITAMIN A DEFICIENCY AS RELATED TO REPRODUCTION IN RANGE CATTLE. Calif. Agr. Expt. Sta. Bull. 560, 30 pp., illus. 1933.

new growth had started; the second was made on September 20 when the grass was in the mature stage, and the third 2 months later, in November, when the plants were dormant and had undergone considerable weathering.

The samples were obtained by clipping the plant about 1½ inches above the ground. Only plants that had not been previously clipped or grazed were collected. The grass was placed in muslin bags and sent to the nutrition laboratory at the University of Arizona to be tested for vitamin A. As soon as it was received there it was dried rapidly by forced ventilation, ground, and all not needed for immediate use was transferred to covered glass jars, and kept in a refrigerator to minimize loss in vitamin content during storage. Tests were begun at once with albino rats which had been depleted of their vitamin A reserves. The method of Sherman and Munsell as modified by Sherman and Burtis [4] was used. It consisted in feeding the grass as the sole source of vitamin A in an otherwise adequate diet to rats whose vitamin A reserves had been exhausted by subsistence upon the same vitamin-A-free ration, and using the growth response as a measure of the concentration of vitamin A in the grass.

TABLE 1.—*Summarized results of feeding range blue grama grass, sampled in 3 consecutive months, to albino rats as the sole source of vitamin A, 1936*

Month of sampling	Amount of glue grama grass in ration	Rats used in test	Average food consumed per day	Average gain or loss in body weight per week	Average survival in test period
	Percent	*Number*	*Grams*	*Grams*	*Days*
August	0.00	6		(1)	32
	.025	4	5.6	(1)	43
	.05	10	8.1	6.5	
	.10	10	9.1	13.3	
	.50	10	9.2	18.0	
	1.00	7	12.3	23.0	
September	.00	4		(1)	
	.05	8	5.4	(1)	35
	.10	8	8.1	5.0	
	.20	8	11.4	10.7	
	.00	5		(1)	28
November	1.00	10	8.3	(1)	41
	2.50	[2] 8	8.8	2.0	(2)
	5.00	12	9.5	6.5	
	10.00	12	13.2	13.3	

[1] Loss.
[2] Three died on forty-fifth day.

In preliminary experiments weighed portions of the dry grass were fed separately. Although the rats readily ate small portions of the young green dry grass cut in August, they found the November grass less palatable and refused large portions of it. For this reason the grass was incorporated in the base ration at a definite percentage level in place of an equivalent percentage of cornstarch. The grass had to be ground very fine to make its separation from the other ingredients of the ration impossible, for there was a marked tendency on the part of the rats to separate out and leave the tougher grass. During the 8-week test period, the rats were housed in individual

[4] SHERMAN, H. C., and BURTIS, P. FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Biol. Chem. 78: 671–680. 1938.

round metal cages with raised screen bottoms. They were allowed to eat ad libitum of the experimental diet (vitamin-A-free in which the grass was incorporated at various percentage levels), and tap water was before them at all times. Careful records of the food consumption and weight of each rat and observations on its general health were made weekly. Autopsies were made on all rats at death or termination of experiment, and all infected lesions and other signs of an inadequate intake of vitamin A were noted.

EXPERIMENTAL DATA

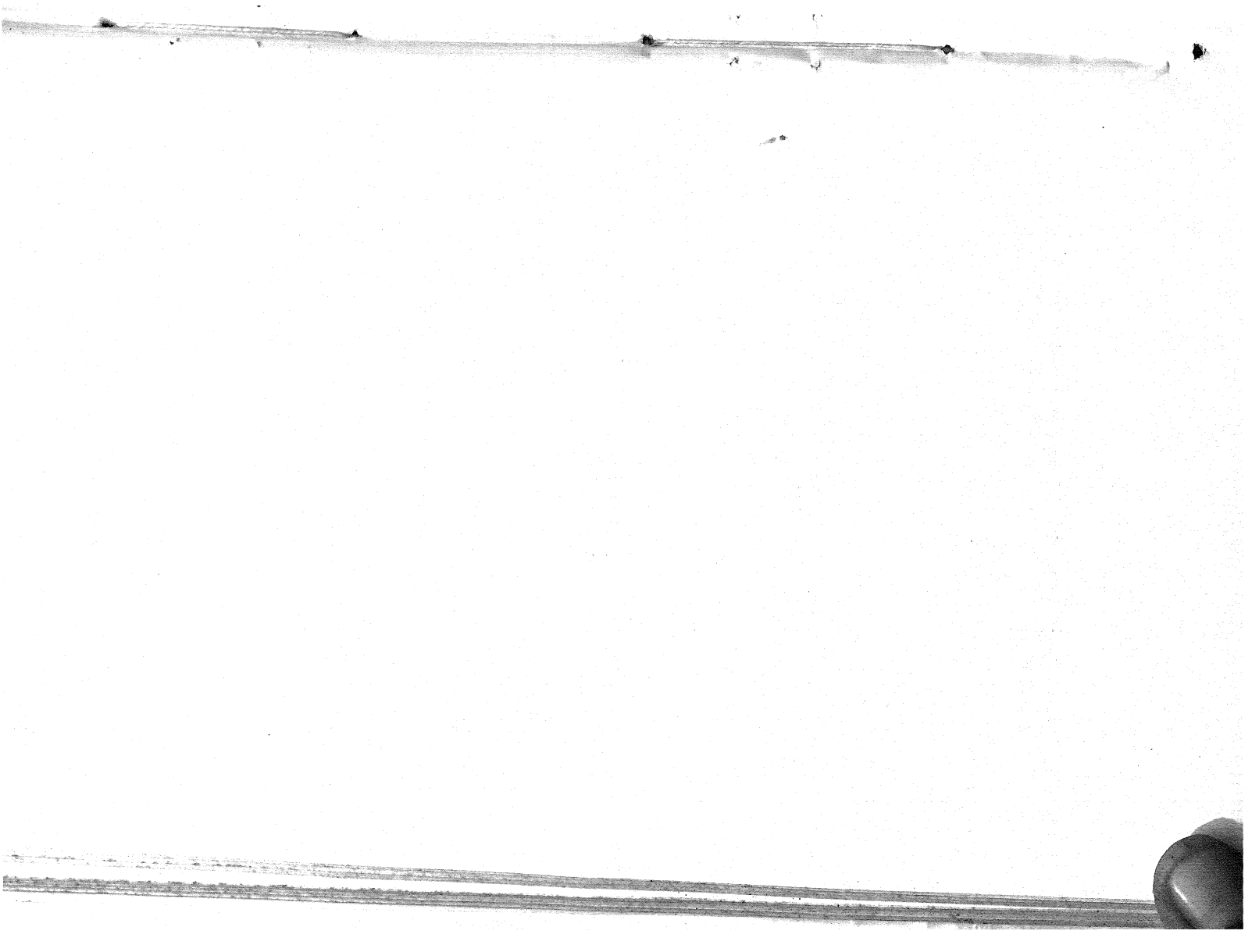
The results of the experiments, summarized in table 1, show that blue grama grass in its early-growth stage is an excellent source of vitamin A. Inclusion of as little as 0.05 percent of the dried grass (approximately 0.004 g daily as computed from food-consumption records) as the only source of vitamin A in a basal vitamin-A-free ration stimulated a rate of growth of approximately 6.5 g per week in the rats subsisting on this diet.

When the same grass cut a month later was tested for its vitamin A content twice as much was required to produce approximately the same rate of gain. The grass was bright green in color, but the blades were tougher and thicker. Blue grama grass at this stage can still be considered a rich source of vitamin A, comparing favorably with the leaves of highest grade alfalfa. By November, however, the blue grama grass had lost, along with its green color, much of its vitamin A. When the November grass was fed as the only source of vitamin A, 5 percent was required to induce an average rate of gain of 6.5 g weekly in the test animals, or 100 times the amount required of the young, tender August grass. Thus a striking deterioration is shown in the nutritive value of the grass in a rather short period of time.

From the foregoing results it appears that blue grama grass is extremely rich in vitamin A at certain stages of its growth, but for the greater part of the year it is relatively deficient in this vitamin. It would seem that range cattle, feeding upon the new growth, would be amply provided with vitamin A, and in fact have access to an amount sufficient to establish a reserve supply. They would, however, be dependent upon this reserve for the greater part of the year because of the striking early decrease in the vitamin A content of their food supply.

SUMMARY

Blue grama range grass at different stages of growth has been tested for vitamin A value by the rat-growth method. When cut in August, only 0.05 percent of the dry grass in a vitamin-A-free ration induced a gain of 6.5 g per week in the rats, thus showing the grass to be an extremely potent source of vitamin A at this stage of its development. Approximately twice as much of the grass cut in September and 100 times as much cut in November was necessary to produce the same rate of gain.



ADAPTATION AND USE OF AUTOMATICALLY OPERATED SAND-CULTURE EQUIPMENT [1]

By H. D. CHAPMAN, *assistant chemist*, and GEORGE F. LIEBIG, JR., *associate in the experiment station, California Agricultural Experiment Station*

INTRODUCTION

For the control of nutrient solution concentration in sand cultures, the automatic periodic flushing system described by Eaton [2] possesses many advantages over the various drip-culture methods. The essential components of a type unit, as described by Eaton, consist of: (1) A large reservoir for the storage of the culture solution, (2) the sand culture, and (3) a motor-driven pump controlled by a time clock. In operation, the sand cultures are periodically flushed with the nutrient solution contained in the reservoirs, the excess solution from the cultures draining by gravity back into the reservoir. The capacity of the reservoir being large as compared with that of the sand cultures, concentrations may be maintained within closely prescribed limits by the addition of water and salts as needed and by occasional renewal.

For the motor-pump unit utilized by Eaton, the authors have substituted a simple glass ejector-type air lift operated by compressed air. By means of a clock-controlled magnetic valve inserted in the air line, the pumps are automatically started and stopped. With an appropriate compressor and storage tank, any number of units can be operated simultaneously. This simple and inexpensive modification is particularly adapted, though not necessarily limited, to greenhouse installations, where space is usually restricted. The present paper gives a brief description of an indoor (2-gallon sand culture) and an outdoor (25-gallon sand culture) set-up, and records certain experiences gained and results secured during the course of more than a year's work.

DESCRIPTION

The general features of the outdoor and indoor equipment are shown in figure 1, and further details of construction are given in figure 2.

AIR LIFT

The glass air lift is composed of three parts: An air inlet tube (*a*), the ejector tube (*b*), and a distributor (*c*), the last named being made from a 500-ml wide-mouthed bottle with the bottom cut off. In operation, air under pressure entering the open glass tube of the ejector member at point *d* lifts the solution into the distributor where it flows by gravity through glass distributor tubes to the sand cultures. Ejector tubes with an inside diameter of 0.5 inch have proved satisfactory for both the small and large units. The compressed-air tube

[1] Received for publication July 6, 1937; issued March 1938. Paper no. 373, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, Calif.

[2] EATON, F. M. AUTOMATICALLY OPERATED SAND-CULTURE EQUIPMENT. Jour. Agr. Research 53: 433-444, illus. 1936.

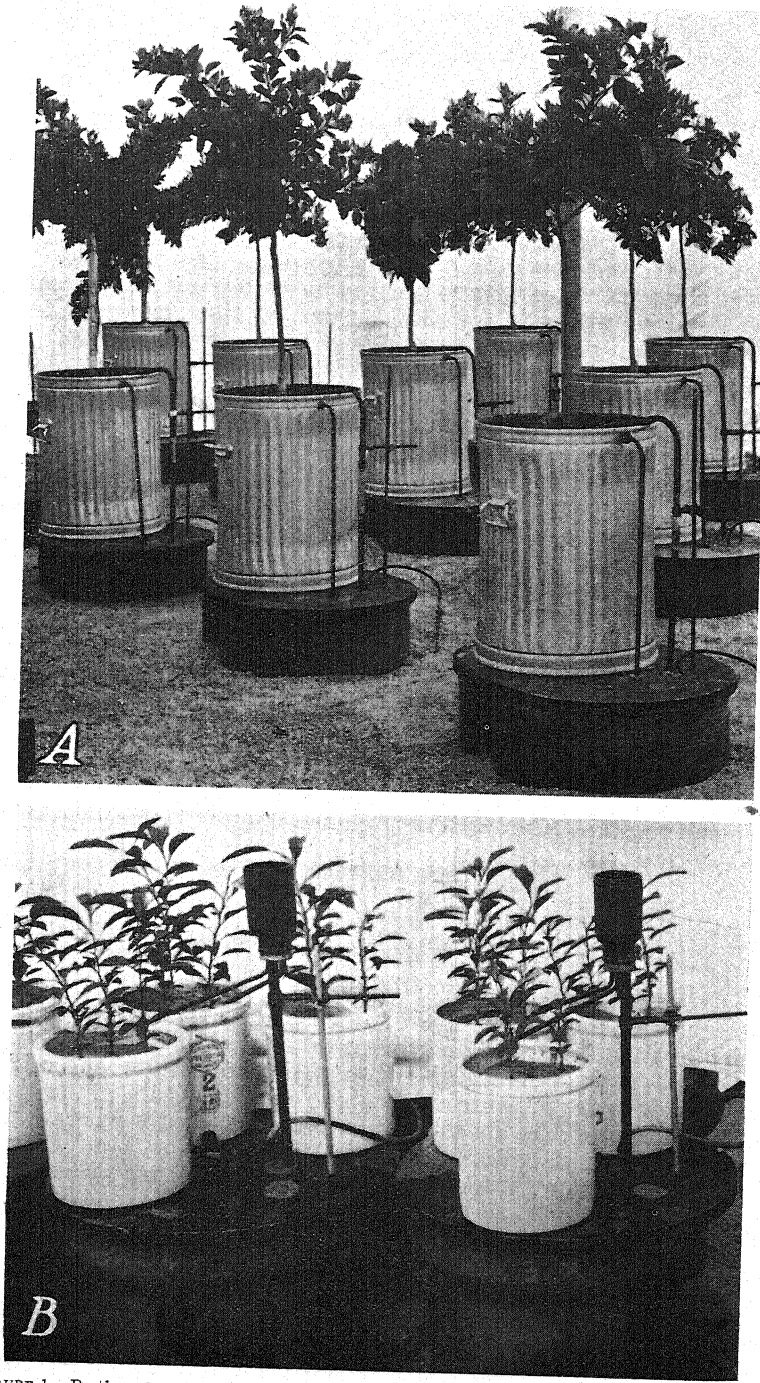


FIGURE 1.—Portion of outdoor (*A*) and indoor (*B*) automatic circulating sand-culture installations.

should discharge at a distance of about 2½ inches above the open end of the ejector tube.

The air pressure required to lift nutrient solution by this method is low, being just slightly in excess of that equivalent to the submergence

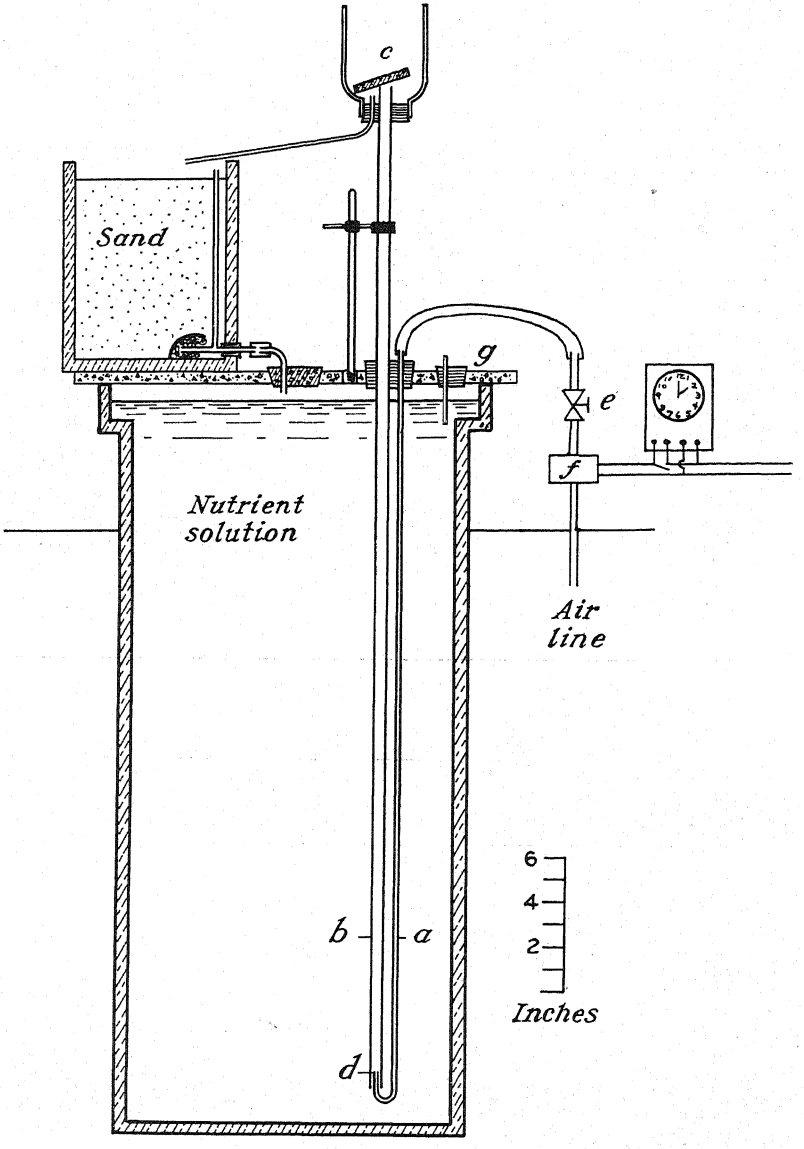


FIGURE 2.—Diagram of indoor unit: *a*, Air inlet tube; *b*, ejector member; *c*, distributor; *d*, point at which air enters *b*; *e*, needle valve; *f*, magnetic valve; *g*, volume indicator.

depth of the ejector tube. The efficiency of pumping depends upon the proportion of the total length of the ejector tube that is submerged. It is obvious that a reservoir cannot be pumped dry by this method. For best results, the submergence depth should be 50 percent or greater.

The rate of pumping can be regulated to some extent by the rate of air flow, and an air valve is required for each unit. (Needle valves are advantageous, but ordinary laboratory air cocks will suffice.) Losses of culture solution from the distributor (*c*) caused by the slight spouting nature of the discharge can be prevented by placing a free-moving cork of large diameter in this vessel.

The rate of pumping should be rapid enough to saturate the sand quickly and to establish free solution above the surface, thus assuring uniform displacement of the solution held by the sand. Overflows must be provided, as it is difficult to adjust the rate of pumping to the rate of percolation through the sand since the latter rate changes somewhat with temperature and stage of plant growth. By trials from time to time, the length of pumping time required to completely replace the old solution held by the sand can be determined. With the particular equipment here described, a 5-minute period for the 2-gallon cultures and a 10-minute period for the 25-gallon cultures is ample.

The volume of air used in relation to the volume of solution pumped and the percentage of ejector submergence for the two types of sand cultures is shown in table 1. At 50-percent submergence a given volume of air will lift a little less than half as much solution. The compressor capacity required for any number of units of size comparable to those herein described can be calculated from these data. If it is desired to operate a large number of units, they can be divided into groups, each group being operated in rotation by a separate time switch and magnetic valve. In this way the size of compressor required may be substantially reduced.

TABLE 1.—*Volume of air used as related to the amounts of solution pumped and percentage of ejection-tube submergence*

Type of sand culture	Height solution pumped	Submergence of ejector tube	Pumping interval	Volume of air used	Volume of solution pumped
	Inches	*Percent*	*Minutes*	*Liters* [1]	*Liters*
2-gallon pot	20	55	5	20.8	10.55
25-gallon garbage can	36	50	10	65.0	28.00

[1] Measured with capillary tube flow meter.

TIME SWITCH AND MAGNETIC VALVES

A simple telecron-motored time switch (G. E. type T–27), previously described by Eaton,[3] has been found satisfactory. Off-and-on riders suitably spaced on a revolving dial make it possible to adjust the time interval between operations to as little as 1 hour and 45 minutes. The actual "on" period can be adjusted from an instant up to about 15 minutes with the "on" riders set at 1 hour and 45 minutes apart.

General controls type K–10 and K–20 magnetic solenoid valves have been in continuous use for over a year and no trouble has been experienced in operating them under air pressures varying from 5 to 30 pounds.

RESERVOIRS

The authors have found that glazed sewer pipes serve especially well as reservoirs. Choice of sizes, durability, low cost, and depth,

[3] EATON, F. M. See footnote 2.

the last named lending itself to efficient pumping by the air lifts, are some of the more important advantages of sewer pipes. For the indoor equipment, sewer pipes with glazed stoppers sealed into the small end were especially made by the manufacturer. In the outdoor equipment, suitable stoppers were sealed into the bell end of the sewer pipe by means of asphaltum [4] prepared for this purpose. Glazed sewer pipes are not entirely impervious, owing to imperfections in the glaze; hence the inside surface should be coated with hot asphaltum or a nontoxic asphalt paint.

Specially made concrete lids 1 inch thick served both as covers for the reservoirs and as supports for the sand cultures. These lids were reinforced with chicken wire and were provided with holes spaced to accommodate the airlift, drainage, and overflow tubes. Pieces of one-eighth inch galvanized-iron pipe screwed into couplings embedded in the cement, and provided with ring-stand clamps, afford a convenient means of support for the airlift.

DRAINS AND OVERFLOWS

With earthenware crocks having a side opening near the bottom, a glass T tube with an extended arm fitted through a tightly fixed rubber stopper, as shown in figure 2, provides for both drainage and overflow. A pad of glass wool over which is placed a cover glass to prevent excessive packing permits free drainage without loss of sand. With the 25-gallon garbage cans a short piece of three-eighths inch galvanized pipe soldered into a hole drilled in the bottom of the can and covered with several layers of graded quartz gravel has provided adequately for drainage. The overflows for these containers are shown in figure 1.

EXPERIMENTAL DATA

IRON SUPPLY

In work with sweet orange (*Citrus sinensis* (L.) Osbeck) seedlings and tomatoes, (*Lycopersicum esculentum* Mill.), the authors have found, in conformity with the experience of Eaton,[5] that finely divided magnetite incorporated with the quartz sand serves as an excellent source of iron when used with slightly acid culture solutions of low phosphate content. On the basis of the authors' experience, it appears likely that by using a sufficient quantity, this source of iron can be utilized under a much wider range of experimental conditions. With 0.1 percent of magnetite in sand containing 2.5 percent of calcium carbonate, cultures of sweet orange seedlings after a time began to display typical iron-deficiency chlorosis. However, when an additional 0.4 percent of magnetite was incorporated with the sand (the chlorotic plants having been temporarily removed), the yellowed leaves began to green up in about 10 days and healthy new shoots subsequently emerged. Control cultures receiving nutrient solution from the same reservoir but not receiving the additional magnetite remained chlorotic. Again, sweet orange seedlings grown in sand cultures containing 0.1 percent of magnetite and supplied with nutrient solution of pH 6.3 and a content of PO_4 maintained at

[4] G–K compound sold by the Atlas Mineral Products Corporation, Mertztown, Pa.
[5] EATON, F. M. See footnote 2.

4 parts per million have shown no symptoms of iron deficiency. However, when the phosphate concentration was raised to 10 parts per million PO_4, some of the plants became chlorotic. By simply increasing the acidity from pH 6.3 to 5.0, the chlorosis disappeared. No doubt, as in the case with the calcium carbonate, an increase in the amount of magnetite would have accomplished the same result.

The authors have found that magnetite fixes phosphate. The aforementioned observations relative to the influence of the concentration of hydrogen ions and of phosphate on the availability of the iron of magnetite is no doubt in part related to phosphate fixation, as well as to the influence of acidity on iron solubility. However, iron availability within the plant is apparently also influenced by acidity and phosphate variations in the nutrient medium.[6] Since the iron is not rendered completely unavailable by virtue of its capacity to fix phosphate, it seems certain that the fixed phosphate must be partly available. The use of magnetite is therefore inadvisable in experiments where the influence of the phosphate level is to be studied. Although subject to this limitation, it appears likely that by utilizing a sufficient quantity of magnetite, this method of supplying iron can be satisfactorily employed under a wide range of experimental conditions. Owing to the variable feeding power and requirements of plants for iron, on the one hand, and the influence of varying nutritive conditions on iron availability, both within and without the plant, on the other, it is advisable to determine by preliminary experiment how much magnetite to incorporate with the sand.

FREQUENCY OF FLUSHING SAND CULTURES

Obviously no given routine can be followed in all cases with regard to the frequency with which cultures must be flushed to assure nutrient control in the root zone. The kind of plant, stage of growth, temperature, light, and humidity, and the moisture-holding capacity of the sand, all enter into this question. In experiments where small sweet orange seedlings were grown in solutions of maintained but very low nitrate levels (0.7 parts per million N as NO^3), three flushings a day (8 a. m., 1 p. m., and 5 p. m.) proved inadequate. When hourly flushings during the daylight hours were adopted, the nitrate-deficiency symptoms exhibited under the three-times-a-day routine disappeared, the plants becoming green and resuming growth. At low nitrate levels, this ion is apparently absorbed relatively more rapidly than water, and a dilution of the solution film is thereby caused in the immediate vicinity of the absorbing surface. Apparently diffusion of nitrate into this zone from points removed is not rapid enough to compensate for the absorption withdrawal. Inasmuch as this observation was made at a time when the citrus plants were small (4 to 5 inches high) and with a plant which grows and absorbs rather slowly, it is apparent that frequent circulation is necessary to maintain the concentration of ions present in great dilution.

Although citrus seedlings are fairly sensitive as regards oxygen requirements in the root medium, the authors have experienced no difficulty with root rotting in cultures flushed at hourly intervals

[6] OLSEN, C. IRON ABSORPTION AND CHLOROSIS IN GREEN PLANTS. Compt. Rend. Lab. Carlsberg, Ser. Chim. 21 (3): 1–52, illus. 1935.
WADLEIGH, C. H., ROBBINS, W. R., and BECKENBACH, J. R. THE RELATION BETWEEN THE CHEMICAL NATURE OF THE SUBSTRATE AND THE DEGREE OF CHLOROSIS IN CORN. Soil Sci. 43: 153–175, illus. 1937.

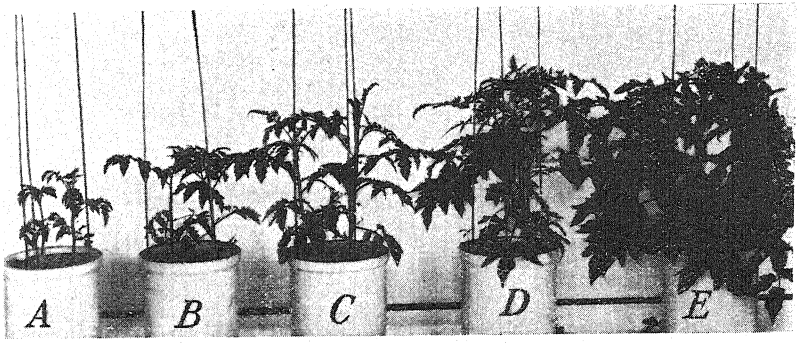


FIGURE 3.—Growth of tomatoes in 2-gallon sand cultures flushed at hourly intervals during daylight with solutions of graded nitrate levels. Maintained at indicated nitrogen levels (±15–20 percent) in parts per million: *A*, 0.14; *B*, 0.70; *C*, 1.4; *D*, 7.0; *E*, 70.0. Seed planted in pots July 3, 1936; plants photographed August 16, 1936.

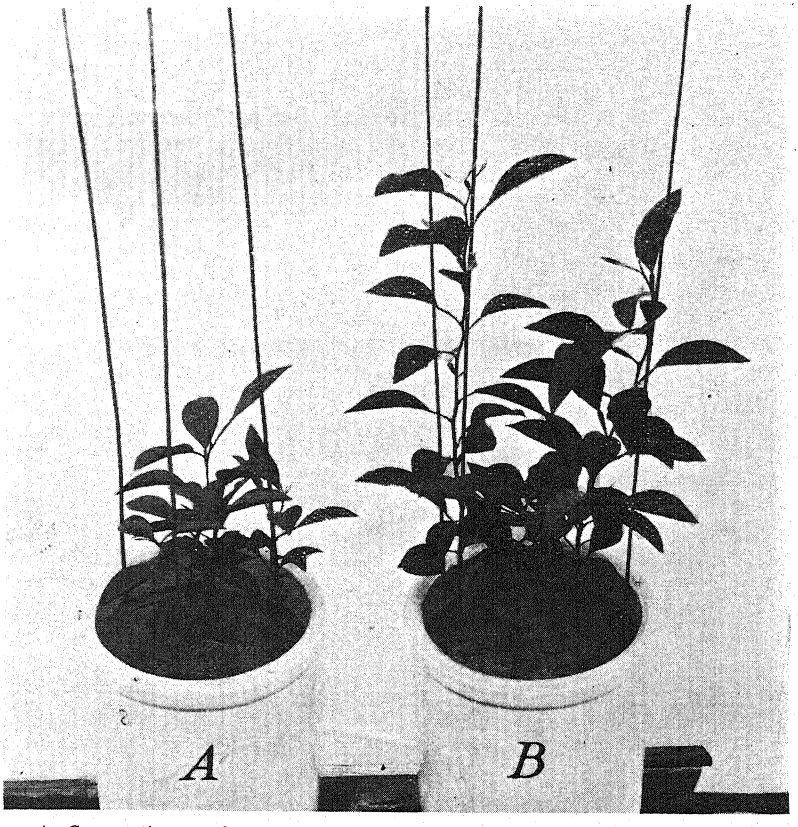


FIGURE 4.—Comparative growth of sweet orange seedlings in (*A*) a well-fertilized loam soil, and in (*B*) a sand culture supplied with a favorable culture solution. Plants in both soil and sand were deep green and healthy, but the growth rate in soil was much slower.

during the 24-hour period, this routine having been followed for months at a time. Obviously, as the excess solution drains from the sand, air takes its place, thus providing effective aeration. The air-lift method of pumping also tends to keep the solution well aerated.

Although compressed air contains traces of oil picked up from the compressor, no evidences of injury to the plants have been noted.

TYPE OF RESULTS SECURED

Both citrus seedlings and tomato plants have made excellent growth in cultures flushed at hourly intervals during the daylight period. Figure 3 shows some results with tomatoes grown in solutions of various nitrate levels.

The comparative growth of sweet orange seedlings during a 3-month period in a well-fertilized loam soil and in a sand culture flushed at hourly intervals during daylight is shown in figure 4. The plants in both the soil and sand were green and healthy, but the growth rate in the soil was much slower than that in the sand.

SUMMARY

Details of two automatically operated sand-culture installations are presented. The nutrient solution is circulated by means of compressed air-operated ejector-type pumps. A clock-controlled magnetic valve in the air line provides for periodic and automatic pumping. This system has given satisfactory service over long periods with little attention other than that required for the maintenance of nutrient-solution concentration. Various experiences relative to iron supply and frequency of flushing are given, together with examples of the excellent results capable of being secured with this type of equipment.

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GROWTH PREDICTION AND SITE DETERMINATION IN UNEVEN-AGED TIMBER STANDS [1]

By WILLIAM A. DUERR, *junior forester*, and S. R. GEVORKIANTZ, *silviculturist*, *Lake States Forest Experiment Station, Forest Service, United States Department of Agriculture*

INTRODUCTION

The prediction of growth in stands of uneven-aged timber has always been a difficult problem in forest measurement. The normal-yield tables that have been developed for nearly every commercial species occurring in even-aged stands have not been adaptable to uneven-aged timber because of too wide variation in the ages of individual trees. For the same reason the evaluation of site in such stands is apt to be unreliable.

For some time growth-prediction methods in use have been based upon the measurement of past diameter growth as revealed by annual rings and the assumption that this growth will be continued at the same rate in the future.[2] These methods are suited only to short-term predictions, and have the further disadvantage that they require an independent estimate of mortality, which is inevitably subject to considerable error.

Permanent sample plots now being established in increasing numbers in all-aged stands will in time yield accurate information on growth. Meanwhile there is a pressing need for a reliable method of growth prediction applicable to the millions of acres of uneven-aged forests now in existence. The northern-hardwood type (sugar maple, yellow birch, hemlock, and associated species) is an important example of these forests, and will be used as an illustration in the method of growth prediction and site determination presented in this paper.

NATURE OF UNEVEN-AGED TIMBER

Undisturbed uneven-aged timber in the Lake States region appears to be composed of an assembly of stands and groups of trees each more or less even-aged. These groups often contain a few scattered individuals considerably older than the rest—remnants of a former stand. Beneath the main body of the stand, in the case of larger timber, may be an understory of newcomers, all considerably younger than the rest. Within each even-aged group, however, the members of the former stand and the younger individuals account for relatively little of the basal area; the majority of trees have evidently originated in a body during the course of a few years.

For example, on the average fifth-acre plot examined in well-stocked stands of mixed hemlock and yellow birch saw timber, the basal area of all trees 1 inch or larger in diameter breast high (d. b. h.)[3] was found to be 38 square feet. Of this, 1 square foot is attributable to the

[1] Received for publication July 23, 1937; issued March, 1938.

[2] CHAPMAN, H. H., and DEMERITT, D. B. ELEMENTS OF FOREST MENSURATION. 452 pp., illus. Albany. 1932. See ch. 24.

[3] 4.5 feet above ground level.

stand of younger individuals and 5 square feet to the former stand, while 84 percent of the total is contributed by the main body of the stand. The trees in this main group range from 6 to 16 inches d. b. h. and from 166 to 182 years. The younger individuals fall entirely in the 2- and 4-inch diameter classes and are all less than 100 years old. The members of the former stand fall in the 16-inch d. b. h. and larger classes and are all older than 200 years. The same type of grouping, occasionally not so pronounced, was found in every stand of northern hardwoods examined for this study.

This division of stands into three age groups is a basic assumption in this method of growth prediction. That it is valid is indicated by several facts: The ages of trees in the younger group almost invariably are arranged about one or two means quite distinct from the average age of the main stand, indicating that these trees have originated in

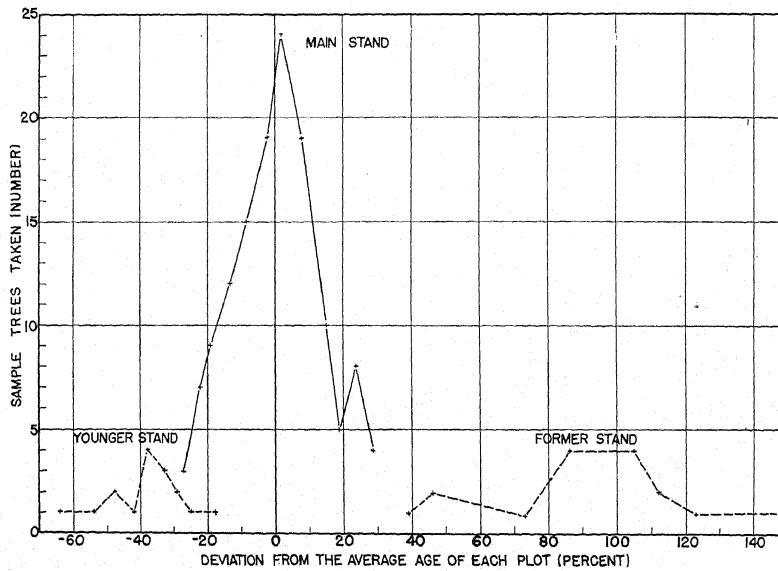


FIGURE 1.—Distribution of sample-tree ages.

groups during periods of a few years, and that these events were definitely subsequent to the establishment of the main stand; the same distinct grouping is found in the members of the former stand. The ages of trees on the average fifth-acre plot, if arranged numerically, appear not to form a continuous series, but to be broken up into groups separated by considerable gaps; furthermore there is almost invariably one group that is decidedly predominant. This condition is illustrated in figure 1, which shows the distribution of ages of 167 sample trees taken on 55 fifth-acre plots in northern-hardwood stands of cordwood size (in which 4-, 6-, and 8-inch trees predominate).

Finally, an examination of stand tables brings out the fact that numbers of main-stand trees by diameter-breast-high classes arrange themselves in a frequency distribution. Members of the younger and former groups show more or less the same tendency, indicating that there are three or more distinct series present. These series, of

course, in an aggregate stand table representing many acres, overlap, and if combined their distribution assumes the typical J-shaped form of uneven-aged stands.

THE FIELD SAMPLE

Circular fifth-acre sample plots were taken throughout Michigan and northern Wisconsin, located mechanically in areas of timber which had not been disturbed by cutting or fire within recent years. Only such undisturbed stands were examined in order that a picture might be obtained of natural developmental trends uninfluenced by either cultural or destructive agencies. The northern-hardwood type was represented by 164 plots. The distribution of these plots by average main-stand diameter classes was as follows:

Average-diameter class, inches	*Plots, Number*	*Average-diameter class, inches*	*Plots, Number*
2	19	14	14
4	40	16	6
6	37	18	0
8	16	20+	3
10	10		
12	19	All	164

On each plot a tally was made of all trees 1 inch or larger in diameter breast high, by species and by the three age groups—younger individuals, main body, and former stand. Judgment of the age group was based upon experience, supplemented by borings for age. Trees were classified "main body" if (1) their ages formed a continuous series about the average; (2) they made up a central group most important from the standpoint of volume; and (3) their ages were set off from those of other trees.

Trees not falling within the limits of this definition were classified as younger individuals or former stand, depending upon their age. Not less than three age-sample trees were taken on each plot, and their species, diameter breast high, age, and age group recorded. Regional volume tables were already available.

ANALYSIS OF THE DATA

MAIN-STAND AGE

In the office the tally on each tally sheet was converted to a per-acre basis; cull trees from this point on were excluded from consideration. The basal area, board-foot volume, and cubic-foot volume per acre represented by the entire tally of sound trees on each sheet were computed. On each sheet were also computed the average stand diameter of the main body, weighted by basal area, and the average age of the main body, as indicated by the age-sample trees. The sheets were then sorted into 2-inch average-diameter classes, and the average age of each class determined. These ages were plotted over their corresponding average diameters, and a smooth curve was drawn through the points (fig. 2, *c*). The resulting values, the average main-

stand ages of northern hardwoods in relation to average main-stand diameters, are as follows:

Average diameter, inches	*Average age, years*	*Average diameter, inches*	*Average age, years*
2	16	14	147
4	28	16	178
6	43	18	209
8	62	20	239
10	86	22	270
12	116	24	301

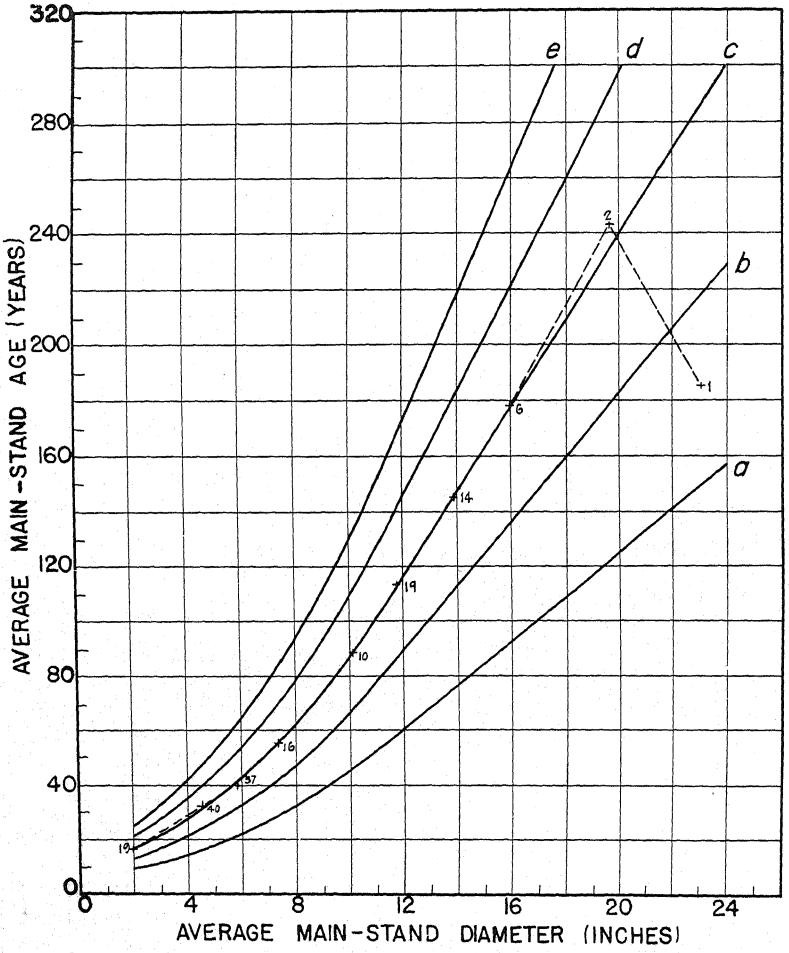


FIGURE 2.—Site-index curves for undisturbed stands of northern hardwoods in Michigan and Wisconsin. In this and subsequent figures 3, 4, 5, 7, and 9 the bases for the plotted points are indicated by numerals.

Such average ages are relative; they express, not the number of years a given stand has been in existence, but the periods of time which that stand has taken to develop from smaller-sized material and the number of years it will require to develop into larger sizes. That is to say, the real significance to be attached to the ages 43 and 116 years for 6-inch and 12-inch stands, respectively, is that it must take about 73 years for stands with a diameter of 6 inches to increase

that diameter to 12 inches. Also, these relative ages are regionwide averages, and are not applicable, therefore, to any specific stands unless those stands happen, by chance, to be average.

BASAL AREA AS AN INDEX OF DENSITY AND YIELD

With these relative ages determined, the next step was to trace the development of stands with increase in the average diameter. Total basal area in square feet per acre was used as a measure of this development. The average basal area per acre was determined for all plots in each average-diameter class, and these basal areas curved over the corresponding average diameters (fig. 3, *b*).

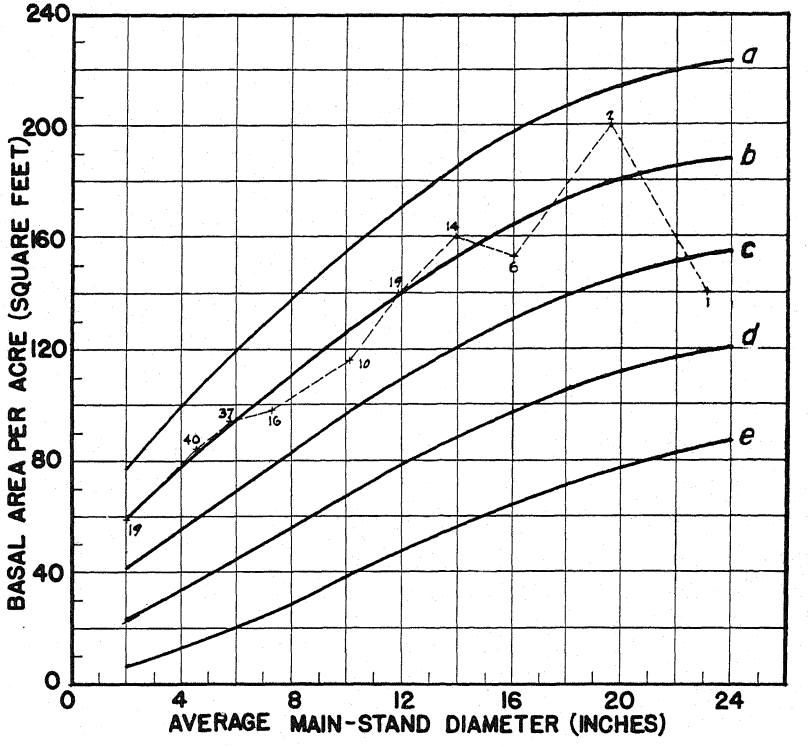


FIGURE 3.—The relation between basal area and average main-stand diameter, five density groups.

This curve represents the way in which basal area increases with the passage of time in an average stand of northern hardwoods, under the fundamental assumption that undisturbed 6-inch stands (for example) will develop during the next 73 years into 12-inch stands essentially similar to the present representatives of the latter diameter class. It is understood, of course, that the field sample gives equal representation to all densities throughout the range of average diameters.

This curve represents the development of stands having an average stocking in basal area, but indicates nothing about the development of well stocked or of poorly stocked stands. The next problem

was to construct curves for the other densities, harmonizing them with the average according to their true relationship to that curve.

In the interpretation of normal-yield tables it is generally assumed that the basal area of an understocked stand will hold a constant percentage relationship to that of the normal stand throughout its development. On the other hand, an allowance is sometimes made for increase in normality—that is, it is sometimes assumed that a young stand with a basal area 50 percent of normal will have a basal area which is 55 or 60 or more percent of normal by the time it reaches maturity. Such an increase in normality would presumably be the result of decreased mortality and increased diameter growth due to an understocked condition.

In order to find the true relationship in this case, the standard deviation in square feet of basal area was calculated for all plots in each diameter class. Since these are standard deviations from different means in the case of each class, they are better expressed as the

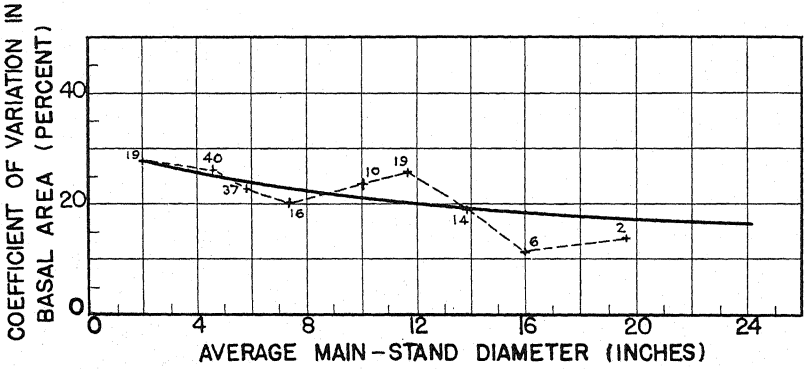


FIGURE 4.—Decrease in coefficient of variation with increasing average main-stand diameter.

coefficient of variation, or the percentage relationship between the standard deviation and its mean. So expressed, they tell a very interesting story.

The average coefficient of variation of northern-hardwood plots belonging to the 2-, 4-, and 6-inch average-diameter classes was found to be 25 percent, meaning that about two-thirds of such plots have basal areas somewhere within the limits of 25 percent more or less than the average. However, plots in the 8-, 10-, and 12-inch diameter classes have a coefficient of variation of 23 percent, and plots with still larger average diameters, only 16 percent. This relationship between the coefficients of variation and their average diameters is expressed in curve form in figure 4.

This appears to mean that the basal area of a poorly stocked stand does not hold a constant percentage relationship to that of the average throughout its development but, on the contrary, gradually approaches the average. It means that the spread of basal areas of individual plots narrows down with increasing average diameter. Poor stocking becomes relatively better, and overstocked stands thin out to some extent. These decreasing coefficients of variation indicate that an increase in normality actually takes place among understocked stands.

These coefficients of variation, then, serve as a basis for constructing more curves of basal area over average diameter above and below the average one already made, in order to express developmental trends in stands of various degrees of stocking (fig. 3). This is done by choosing some convenient arbitrary percentage of the coefficient of variation and using it to fix the interval between successive density curves. In this case, 110 percent of the coefficient of variation was used to determine the position of four new curves of basal area over average diameter, one above and three below the average. This is an adapta-

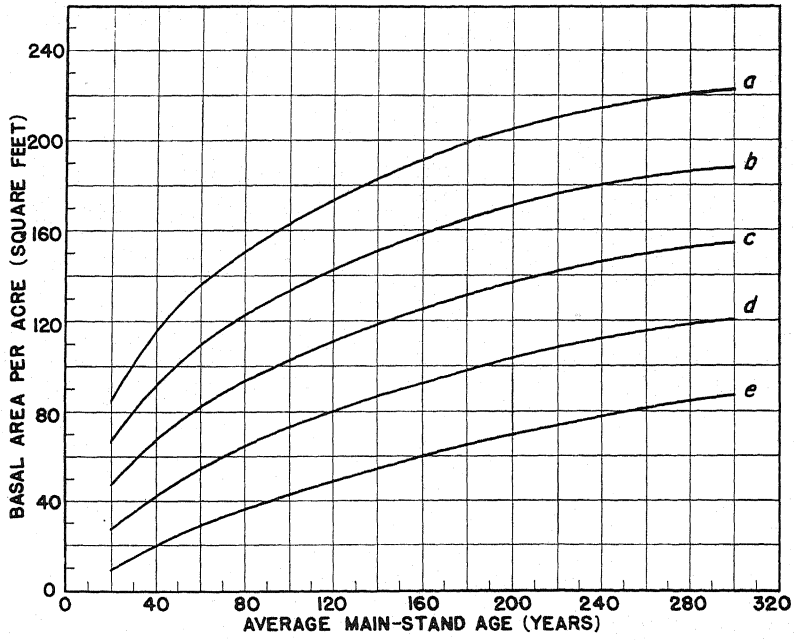


FIGURE 5.—Basal-area growth curves, showing the relationship between basal area and age for five density groups.

tion of the method of constructing harmonized curves described by Osborne and Schumacher.[4]

It is now a simple matter to construct a series of curves of basal area over age (basal-area growth curves). Figure 3 presents the relation of basal area to average diameter; figure 2, c, the relation between age and average diameter for average stands. Figure 5 shows the result of combining these two sets of relationships, thereby eliminating the factor of average diameter common to both.

In table 4 under site index 3, these growth curves are expressed in the form of a growth table. Again, it must be borne in mind that the growth values so far obtained are applicable only to regionwide average stands, and not to any specific stands unless they happen by chance to be average.

The question now arises: Are these basal-area growth values, based as they are upon undisturbed stands, applicable to the general run of

[4] OSBORNE, J. G., and SCHUMACHER, F. X. THE CONSTRUCTION OF NORMAL-YIELD AND STAND TABLES FOR EVEN-AGED TIMBER STANDS. Jour. Agr. Research 51: 547-564, illus. 1935.

stands found throughout a region? The curves for poor density represent what happens to stands that by nature are poorly stocked. Do they also represent what happens to stands poorly stocked as the result of cutting, fire, or some other disturbance?

Growth in basal area is a function of (1) diameter growth and (2) mortality. Unfortunately there are no reliable data available for comparing the mortality in undisturbed stands with that in cut stands. It is possible that mortality rates might be slightly higher in cut stands as a result of disturbance; at any rate, whatever difference exists would probably tend to make growth predictions based on the undisturbed curves slightly liberal when applied to cut stands.

In the case of current growth in diameter a positive check was possible. Increment borings were taken in the field on the undisturbed plots, and borings from cut-over plots taken in other studies were also available. According to these data, half-normal undisturbed stands (considering density 1 as normal) are growing 1.40 times as fast in diameter as normal stands; stands half-normal as the result of cutting are growing nearly 1.45 times as fast. In the case of quarter-normal stands the values are 1.82 and 1.86 for undisturbed and cut stands respectively. Actually these differences are not significant for the size of the sample taken; but they mean, if anything, that the undisturbed-growth curves may be slightly conservative in respect to diameter growth.

It is possible, then, by means of the basal-area growth tables, to predict with some degree of assurance the future basal area and the growth in basal area for different periods of time for any average stand whose age and present basal area per acre are known. It remains only to translate these growth-table values into terms of volume.

This may be done by using the ratio between volume and basal area.

RATIO OF VOLUME TO BASAL AREA

In young stands the ratio of volume—board-foot volume, for example—to basal area is small. This is because few trees have yet reached a size sufficiently large to be given any board-foot volume at all, while there is already considerable basal area. As the stand develops and many trees begin to grow to merchantable size, the board-foot volume increases rapidly. At the same time the basal area is increasing, but at a slower rate, so that the ratio of board-foot volume to square feet of basal area grows rapidly larger. In this way the number of board feet per square foot in a stand increases steadily with age. The increase of the ratio of volume to basal area reflects stand development in terms of the unit of volume employed. It is most rapid at the time when many trees are just coming into merchantability. As time goes on, this effect is less pronounced, and the ratio increase slacks off.

The ratio of volume to basal area also reflects stand composition with regard to the relative numbers of trees of various sizes, an important consideration in dealing with uneven-aged stands. A small ratio indicates the presence in the stand of a large proportion of small trees having considerable basal area but little volume; a large ratio indicates a predominance of large diameters in the stand. Thus increase in ratio during a given period is closely correlated with the

ratio itself at the beginning of the period—that is, future ratio is correlated with present ratio.

For this reason it was not possible to convert the relationship between basal area and age into a relationship between volume and age which might be applied to all stands. A certain volume per acre in one stand may be associated with a high basal area, indicating that many small trees are present and that a large volume growth may be expected; the same volume per acre in another stand of the same age may be associated with a relatively small basal area, indicating that growth possibilities are comparatively limited. Thus in average uneven-aged stands, volume yields are controlled independently by

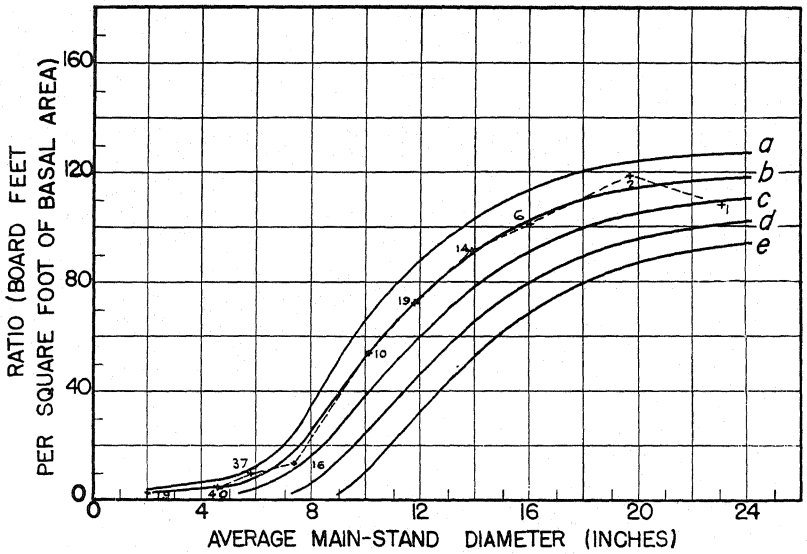


FIGURE 6.—The relationship between the board-foot square-foot ratio and average main-stand diameter, by five ratio groups.

(1) basal area and (2) the ratio of volume to basal area, and some means must be found for constructing ratio growth tables as well as basal-area growth tables.

The same method may be used for constructing such ratio growth tables as was used for making the basal-area tables. The ratio of board-foot volume to basal area, for example, was computed on each tally sheet. This volume, incidentally, is gross Scribner log measure, neither woods cull nor mill cull having been deducted. The ratios so computed were averaged by average-diameter classes, and the averages were used in the construction of a curve showing the relationship between the board-foot square-foot ratio and average diameter (fig. 6, *b*).

The coefficient of variation of the ratios of individual plots within each average-diameter class was then computed, curved (fig. 7), and used to construct additional curves of ratio over average diameter in the same way that the basal-area curves were made.

Finally the relation between age and average diameter (fig. 2, *c*) was used to establish the relationship between ratio and age. (See fig. 8 and table 5.)

Ratio tables for cubic-foot volume were made in the same way (table 6).

The final procedure, then, for predicting the future volume—e. g., board-foot volume at the end of 40 years—of an average stand is as follows: Determine the present board-foot volume and basal area per acre, and from these the present ratio; determine the age of the stand, based on the ages of main-stand trees. From the basal-area growth table find the basal area 40 years hence. From the ratio table find the

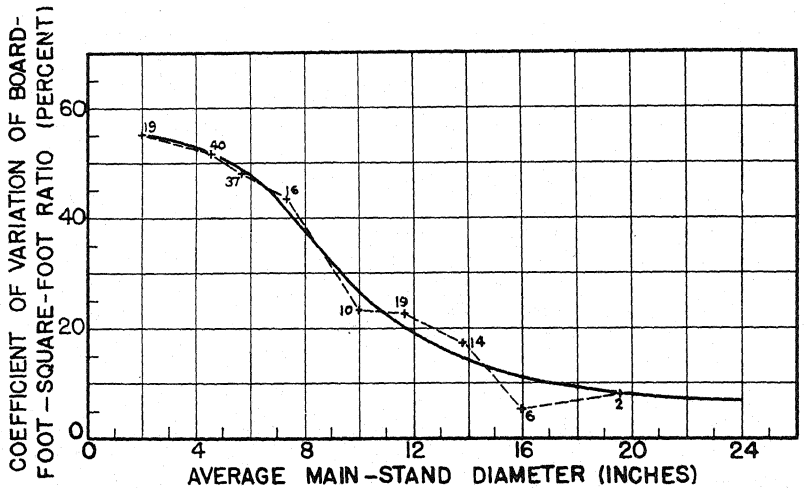


FIGURE 7.—The correlation between coefficient of variation of the board-foot square-foot ratio and average main-stand diameter.

ratio 40 years hence. Multiply the 40-year basal area by the 40-year ratio. The result is the yield in board-foot volume at the end of 40 years.

SITE

One factor—site—remains to be considered. Thus far this method of growth and yield prediction is applicable only to regionwide average stands. How may it be made applicable to individual stands whose sites may differ from the average?

The key to this problem seems to lie with the ages of plots within the various average-diameter classes. The average age of plots in the 8-inch diameter class was found to be 62 years, but the deviation of individual plot ages from this average was considerable. Expressed as the coefficient of variation, it amounted to 29.5 percent of the average. This value of 29.5 percent, moreover, was obtained independently for each of the three average-diameter groups mentioned in the discussion of basal areas, indicating that the standard deviation in age of individual plots holds a constant percentage relationship to its mean, regardless of size.

This is quite a different situation from that found in the case of basal area. With basal area there was a gradual approach toward the average as stands matured; with age there appears to be a factor at work which makes some stands younger, some older than the average for that size; and further than this, as these stands develop, their

variation from the average appears to increase in direct proportion to the length of time involved. In other words, there is a factor which causes some stands to reach a given size much sooner than others, and this factor is cumulative and constant with the passage of time.

Whether this is a single factor or several factors working together, or whether it is termed "site quality" or something else, is not important. For the sake of simplicity it will be so termed here, and defined thus: Site quality is an expression of those natural elements in the habitat which combine to control the rate of increase in the average diameter of a timber stand. According to this definition, if the average site will produce an 8-inch stand in 62 years, then sites producing 8-inch stands in, for example, 32 years should be called good; those requiring 92 years to achieve the same result should be called

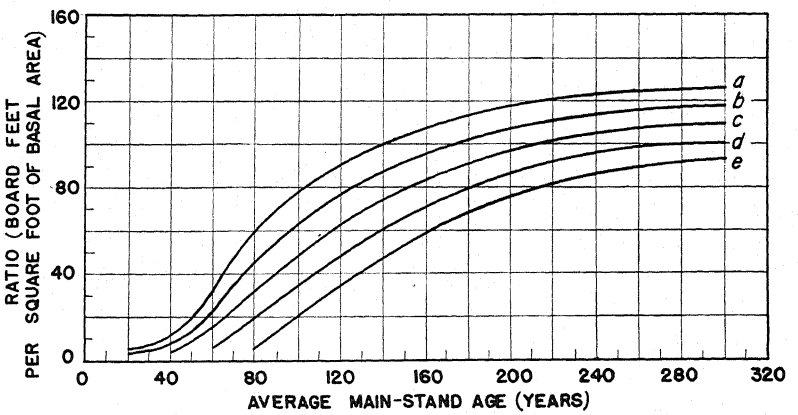


FIGURE 8.—The board-foot square-foot ratio curved over age; five ratio groups.

poor. Furthermore, the range between 32 and 92 may be divided into a series of site classes, from the best to the poorest, and a similar series may be established for each of the average-stand-diameter classes.

This has been done in figure 2, which expresses site quality in terms of average main-stand diameter and average main-stand age for the northern-hardwood type. Site 3, or the average, was represented by the original curve *c* of age over diameter. The other site-index curves were harmonized with the average according to the same method used for basal area and for the board-foot square-foot ratio. The interval employed in this case was 85 percent of the coefficient of variation, giving a convenient spread between site 1 and site 5.

It is, of course, obvious that the definition of site quality expressed in figure 2 is applicable only to stands of northern hardwoods which have not been disturbed by either cultural or destructive agencies. These curves are expressed in tabular form in table 3.

It is now a simple matter to construct basal-area growth curves and ratio-growth curves for sites other than the average. A set of basal-area growth curves for site 1 may be obtained from the curves in figure 5 (representing site 3) by substituting for the site 3 age values the corresponding values read from curve *a* in figure 2. This new age scale would read 10 years at the point now marked 19; 20 years instead of the present 39; 30 years instead of 58; and so forth, indicat-

ing that the same result is achieved in 10 years on site 1 that requires 19 years on site 3, and so on. A set of basal-area and of ratio curves was obtained for each of the five sites, and these curves converted to table form (tables 4, 5, and 6).

STATISTICAL BASIS

The justification for using these data to determine site and predict growth in a stand of northern hardwoods is based upon certain relationships capable of being tested statistically. Such tests were actually carried out, and the following facts established regarding the basic data:

(1) There is no correlation between the density index of plots and their site index. That is, the variation in basal area from the basal-

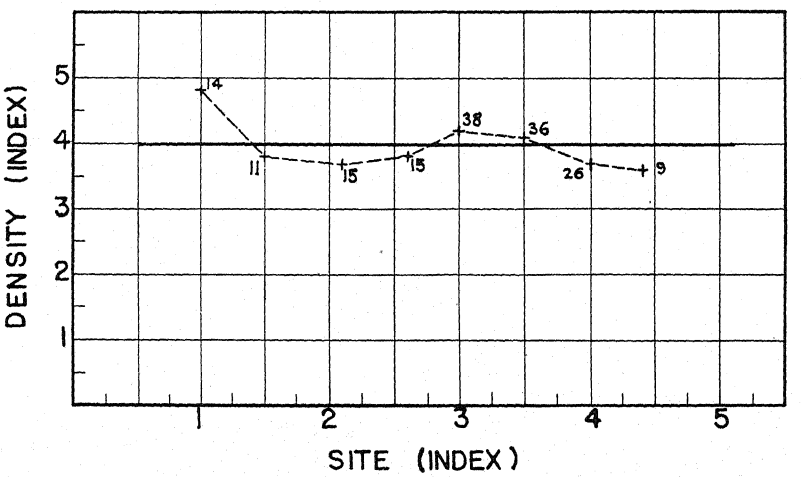


FIGURE 9.—Lack of correlation between the density index of plots and their site index.

area average-diameter curve is not related to age. This, in turn, means that the use of a single basal-area average-diameter curve for all site indices is well justified.

(2) There is good correlation between the age of main-stand sample trees and the average diameter of the plots on which they were taken (correlation coefficient 0.819; 492 cases).

The method of carrying out test No. 1 is illustrated in figure 9.

Test No. 2 was carried out by running a partial correlation, using the variables age, average diameter, and density. For this purpose the range of densities represented in figure 3 was further divided to allow for a classification into 21 density groups.

The variation in age of the main stand in the northern-hardwood type depends upon three conditions: (1) The stage of stand development—i. e., whether the stand has a large or small average diameter; (2) site, or variation in place, revealed by the age variation between plot means; and (3) the age variation among individual main-stand sample trees within a plot, determined by the classification of main-stand trees used in the field (pp. 81 to 83). These values are presented in table 1.

TABLE 1.—*Variation in main-stand age, by size classes*

Variation	Mean variance in age by main-stand size class—		
	1	2	3
	Years	*Years*	*Years*
Between plots (V_s^2)	563.68	5,749.25	7,437.98
Between plot means (σ_s^2)	165.48	1,758.61	1,862.64
Within plots (σ_r^2)	67.24	473.42	1,850.06

The mean variance in age increases with the size class of stands. Size-class 1 represents stands with average main-stand diameters of 2 through 6 inches; size-class 2, stands with average diameters of 8 through 12 inches; size-class 3, 14 inches or more.

The variance in age between plots (V_s^2) is made up of two components, namely, variance between plot means (σ_s^2) and variance within plots (σ_r^2). That is to say,

$$V_s^2 = n\sigma_s^2 + \sigma_r^2,$$

where n equals the number of age borings per plot.

The variance between plots is large and is significantly different from the variance within plots, in all stands, as determined by Fisher's Z test.[5] Most of the variance between plot means is due to site differences.

The square of the standard error of the grand mean [6] can be expressed as

$$\sigma_M^2 = \frac{1}{mn}(n\sigma_s^2 + \sigma_r^2), \text{ o}$$

$$\sigma_M^2 = \frac{1}{m}\left(\sigma_s^2 + \frac{\sigma_r^2}{n}\right),$$

where m equals the number of plots taken in each size class, and n equals the number of age borings taken on each plot. In other words, the standard error of the grand mean depends not only upon the two variances (σ_s^2 and σ_r^2), but also upon the number of borings per plot and especially upon the number of plots taken within a given size class.

However, in the case of stands homogeneous in respect to site and average diameter, since σ_s here equals zero, the square of the standard error of the mean age can be expressed in a simpler form—

$$\sigma_M^2 = \frac{\sigma_r^2}{mn}.$$

That is, the standard error here will depend upon the variance within plots and the total number of borings (mn) used.

Table 2 shows how the standard error of age determinations varies with the total number of borings taken, or mn. If, for example, a cruise based on fifth-acre sample plots employs altogether 10 acres of

[5] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 6, rev. and enl., 339 pp., illus. Edinburgh [etc.] 1936.

[6] TIPPETT, L. H. C. THE METHODS OF STATISTICS; AN INTRODUCTION MAINLY FOR WORKERS IN THE BIOLOGICAL SCIENCES. 222 pp., illus. London. 1931. See pp. 93 and 176. Tippett's notation has been used throughout this paper.

plots, 50 sample plots in all are taken. If, furthermore, 2 borings are taken on each plot, there will be 100 borings. With this number of borings, the average age of stands of northern hardwoods of size class 2 (growing on all sites) will be estimated with a standard error of 6.3 years, an accuracy adequate for the uses to which the estimated average age is to be put—growth prediction and site determination. Should the stands show no marked differences in site or average diameter, the same 100 borings would estimate the mean age within 2.2 years, on the average.

TABLE 2.—*Standard error of mean-age determinations in relation to size class and number of borings used (all sites combined)*

Plots used (m)	Borings per plot (n)	Total age borings (mn)	Standard error (σ_M) by main-stand size class—			Plots used (m)	Borings per plot (n)	Total age borings (mn)	Standard error (σ_M) by main-stand size class—		
			1	2	3				1	2	3
Number	*Number*	*Number*	*Years*	*Years*	*Years*	*Number*	*Number*	*Number*	*Years*	*Years*	*Years*
10	3	30	4.3	13.8	15.7	50	2	100	2.0	6.3	7.5
10	4	40	4.3	13.7	15.2	50	3	150	1.9	6.2	7.0
10	6	60	4.2	13.6	14.7	50	4	200	1.9	6.1	6.8
20	4	80	3.0	9.7	10.8						

Incidentally, the standard errors of age determination for the northern-hardwood plots used as the basis for this study were as follows: For plots belonging to size-class 1, 1.4 years; size-class 2, 6.5 years; size-class 3, 10.4 years. These standard errors represent about 6 percent of the average ages of the groups of plots involved.

GROWTH TABLES AND THEIR APPLICATION

The method of growth prediction and site determination for uneven-aged timber stands can now be put into practice, using four basic tables, taken from the curves already presented (tables 3, 4, 5, and 6).

TABLE 3.—*Site-index*[1] *table: Basic values for determining site index of a stand whose average diameter and age are known*

[Northern hardwoods—Lake States]

Average main-stand diameter	Base age	Age interval	Average main-stand diameter	Base age	Age interval	Average main-stand diameter	Base age	Age interval
Inches	*Years*	*Years*	*Inches*	*Years*	*Years*	*Inches*	*Years*	*Years*
2.0	4	4	9.5	22	19	17.0	54	46
2.5	5	5	10.0	24	21	17.5	56	48
3.0	6	5	10.5	26	23	18.0	59	50
3.5	7	6	11.0	28	24	18.5	61	52
4.0	8	7	11.5	31	26	19.0	63	54
4.5	9	7	12.0	33	28	19.5	65	56
5.0	10	8	12.5	35	30	20.0	67	57
5.5	11	9	13.0	37	32	20.5	69	59
6.0	12	10	13.5	39	34	21.0	71	61
6.5	13	11	14.0	41	35	21.5	74	63
7.0	14	12	14.5	43	37	22.0	76	65
7.5	16	14	15.0	45	39	22.5	78	67
8.0	17	15	15.5	48	41	23.0	80	68
8.5	18	16	16.0	50	43	23.5	82	70
9.0	20	18	16.5	52	44	24.0	84	72

[1] Site index $= \frac{\text{(average main-stand age)} - \text{(base age)}}{\text{(age interval)}}$

TABLE 4.—*Growth table: Total stand basal area per acre* [1] *in relation to age, site, and density*

[Northern hardwoods—Lake States]

Main-stand age (years)	Site index 1					Site index 2					Site index 3				
	Density index 1	Density index 2	Density index 3	Density index 4	Density index 5	Density index 1	Density index 2	Density index 3	Density index 4	Density index 5	Density index 1	Density index 2	Density index 3	Density index 4	Density index 5
	Sq. ft.	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*
20	114.2	90.1	66.0	42.0	17.9	95.4	74.3	53.2	32.1	11.0	83.5	64.8	46.0	27.2	8.5
40	146.8	118.7	90.6	62.4	34.3	128.6	102.6	76.6	50.7	24.7	115.2	91.2	67.2	43.1	19.1
60	170.3	139.6	109.0	78.4	47.7	149.4	120.9	92.4	63.8	35.3	135.7	108.8	81.9	55.0	28.1
80	188.8	156.3	123.8	91.3	58.8	165.0	134.8	104.5	74.2	44.0	149.9	121.4	92.8	64.3	35.8
100	203.1	169.4	135.6	101.8	68.1	178.2	146.6	115.0	83.5	51.9	162.0	132.1	102.2	72.4	42.5
120	212.3	178.2	144.1	110.0	75.9	189.7	157.0	124.2	91.5	58.8	172.9	141.8	110.8	79.7	48.6
140	219.1	184.9	150.6	116.4	82.2	199.4	165.8	132.3	98.8	65.2	182.6	150.5	118.4	86.4	54.3
160	222.9	188.9	154.9	120.9	86.9	207.3	173.2	139.0	104.9	70.8	191.4	158.4	125.4	92.5	59.5
180	------	------	------	------	------	213.4	179.1	144.8	110.4	76.1	198.7	165.1	131.6	98.0	64.4
200	------	------	------	------	------	218.1	183.8	149.4	115.1	80.8	204.8	170.9	137.0	103.1	69.2
220	------	------	------	------	------	221.4	187.4	153.4	119.3	85.3	209.8	175.8	141.7	107.6	73.6
240	------	------	------	------	------	------	------	------	------	------	214.1	179.9	145.8	111.6	77.4
260	------	------	------	------	------	------	------	------	------	------	217.5	183.3	149.1	114.9	80.7
280	------	------	------	------	------	------	------	------	------	------	220.3	186.2	152.2	118.1	84.0
300	------	------	------	------	------	------	------	------	------	------	222.6	188.7	154.8	121.0	87.1

Main-stand age (years)	Site index 4					Site index 5				
	Density index 1	Density index 2	Density index 3	Density index 4	Density index 5	Density index 1	Density index 2	Density index 3	Density index 4	Density index 5
	Sq. ft.	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*
20	75.8	58.4	41.0	23.6	6.2	70.0	53.5	37.0	20.4	3.9
40	104.6	82.1	59.6	37.1	14.6	97.4	76.0	54.6	33.2	11.8
60	124.8	99.2	73.6	47.9	22.3	115.7	91.4	67.2	43.0	18.7
80	139.3	111.8	84.4	57.0	29.5	129.7	103.5	77.3	51.1	24.9
100	150.6	122.0	93.3	64.6	36.0	140.7	113.2	85.7	58.2	30.7
120	160.2	130.6	100.9	71.2	41.6	150.2	121.6	93.1	64.6	36.0
140	169.2	138.6	107.9	77.2	46.6	158.6	129.2	99.7	70.2	40.8
160	177.7	146.1	114.5	82.9	51.3	166.2	136.0	105.7	75.4	45.2
180	185.3	153.0	120.6	88.2	55.9	173.3	142.2	111.2	80.1	49.0
200	191.9	158.9	126.0	93.0	60.0	179.9	148.1	116.2	84.4	52.6
220	197.9	164.4	131.0	97.5	64.0	186.2	153.7	121.2	88.6	56.1
240	203.0	169.2	135.4	101.5	67.7	192.1	159.0	125.8	92.6	59.5
260	207.3	173.2	139.2	105.1	71.0	197.4	163.8	130.2	96.5	62.9
280	210.9	176.7	142.5	108.3	74.1	202.1	168.2	134.2	100.2	66.3
300	214.1	179.8	145.6	111.3	77.0	206.4	172.2	138.0	103.8	69.6

[1] Trees 1 inch and more in diameter breast high; cull trees excluded.

Take as an example a 100-acre stand of northern hardwoods in eastern Wisconsin. A 10-percent inventory cruise is to be run, using fifth-acre plots. A prediction is required of the annual growth in board feet for the next 20 years.

An accuracy of age determination representing a standard error of about 6 years is desired. A cursory inspection shows that the timber all falls in size-class 2; table 2 shows that little increased accuracy is to be obtained from numerous borings on each plot, about two borings per plot probably being adequate. The cruise is run, the data com-

piled in the office, and the following figures obtained (excluding cull trees):

(1) Average volume per acre: 8,250 board feet (Scribner).
(2) Average basal area per acre: 150.0 square feet.
(3) Board-foot square-foot ratio: 55.0.
(4) Average main-stand diameter: 9.2 inches.
(5) Average main-stand age: 42 years.

Table 3 indicates that site index is $\frac{42-21}{18}$, or 1.2, called 1 (excellent).

Table 4, site index 1, 42 years, places 150.0 square feet nearest density index 1 (fully stocked). The increase in basal area during the next 20 years (from age 42 to age 62) for density index 1, site 1, is, by simple interpolation,

$$\left[\frac{18}{20}(170.3-146.8)\right]+\left[\frac{2}{20}(188.8-170.3)\right],$$

or 23.0 square feet. The basal area to be expected 20 years hence is then 150.0+23.0=173.0 square feet per acre.

TABLE 5.—*Growth table: ratio (board feet per square foot of basal area)*[1] *in relation to age, site, and ratio group*

[Northern hardwoods—Lake States]

Main-stand age (years)	Site index 1					Site index 2					Site index 3				
	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*
20	9.6	6.2	2.8	------	------	5.8	3.7	------	------	------	4.5	2.8	------	------	------
40	55.6	42.5	29.4	16.3	3.2	21.0	14.8	8.5	2.3	------	9.6	6.2	2.9	------	------
60	88.2	74.0	59.8	45.6	31.4	58.3	44.8	31.4	17.9	4.4	31.8	23.0	14.1	5.3	------
80	106.2	93.6	81.1	68.6	56.0	80.8	66.5	52.2	37.8	23.5	58.6	45.3	32.0	18.7	5.4
100	116.8	106.0	95.3	84.6	73.8	96.7	83.0	69.4	55.8	42.1	77.5	63.0	48.6	34.2	19.7
120	122.8	113.4	104.1	94.8	85.4	107.7	95.2	82.6	70.1	57.6	90.5	76.5	62.4	48.4	34.4
140	126.0	117.4	108.8	100.2	91.6	115.4	104.1	92.8	81.5	70.2	100.4	87.2	74.0	60.8	47.6
160	127.1	118.8	110.4	102.1	93.8	120.3	110.2	100.1	90.0	79.9	108.2	95.9	83.6	71.4	59.1
180	------	------	------	------	------	123.6	114.4	105.2	95.9	86.7	113.9	102.6	91.4	80.2	68.9
200	------	------	------	------	------	125.7	117.0	108.3	99.6	90.9	118.2	107.8	97.4	87.0	76.6
220	------	------	------	------	------	126.7	118.4	110.0	101.6	93.3	121.4	111.7	102.0	92.3	82.6
240	------	------	------	------	------	------	------	------	------	------	123.9	114.8	105.6	96.5	87.4
260	------	------	------	------	------	------	------	------	------	------	125.5	116.8	108.0	99.2	90.5
280	------	------	------	------	------	------	------	------	------	------	126.6	118.1	109.6	101.1	92.6
300	------	------	------	------	------	------	------	------	------	------	127.1	118.8	110.4	102.1	93.8

Main-stand age (years)	Site index 4					Site index 5				
	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*
20	4.0	2.6	------	------	------	3.8	2.4	------	------	------
40	7.0	4.3	------	------	------	5.8	3.8	------	------	------
60	16.0	11.2	6.3	------	------	10.0	6.6	3.1	------	------
80	38.1	27.8	17.6	7.3	------	22.2	15.6	9.1	2.5	------
100	59.8	46.3	32.8	19.3	5.8	42.0	31.0	19.9	8.9	------
120	74.5	60.0	45.4	30.9	16.4	59.9	46.3	32.8	19.2	5.6
140	86.2	71.8	57.4	42.9	28.5	72.8	58.4	44.1	29.8	15.4
160	95.2	81.5	67.8	54.0	40.3	83.0	68.6	54.2	39.9	25.5
180	102.7	89.8	76.8	63.8	50.9	91.6	77.5	63.4	49.4	35.3
200	108.9	96.7	84.6	72.4	60.2	98.6	85.1	71.6	58.1	44.6
220	113.8	102.3	90.8	79.4	67.9	104.4	91.5	78.6	65.7	52.8
240	117.2	106.4	95.7	85.0	74.2	109.2	97.0	84.8	72.6	60.4
260	119.9	109.8	99.6	89.5	79.4	113.1	101.5	90.0	78.4	66.8
280	122.1	112.6	103.0	93.5	84.0	116.3	105.4	94.4	83.4	72.5
300	124.0	115.0	106.0	96.9	87.9	118.7	108.4	98.2	87.9	77.6

[1] Scribner scale, gross. Trees 9 inches and more d. b. h., to a variable top diameter, the minimum being 8 inches inside bark (6 inches for conifers); cull trees excluded.

TABLE 6.—*Growth table: Ratio (cubic feet per square foot of basal area)* [1] *in relation to age, site, and ratio group*

[Northern hardwoods—Lake States]

Main-stand age (years)	Site index 1					Site index 2					Site index 3				
	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*
20	13.4	10.3	7.2	4.2	1.1	9.5	7.1	4.8	2.4	-----	7.7	5.7	3.8	1.8	-----
40	22.8	18.7	14.6	10.6	6.5	17.4	13.7	10.0	6.4	2.7	13.5	10.4	7.3	4.2	1.1
60	26.8	23.0	19.2	15.3	11.5	23.2	19.1	15.0	10.9	6.8	19.4	15.5	11.6	7.7	3.8
80	28.7	25.3	21.8	18.4	15.0	26.0	22.0	18.1	14.2	10.2	23.3	19.2	15.2	11.1	7.0
100	29.7	26.6	23.5	20.4	17.3	27.7	24.0	20.4	16.7	13.0	25.6	21.6	17.6	13.7	9.7
120	30.2	27.3	24.4	21.6	18.7	28.7	25.3	22.0	18.6	15.2	27.0	23.2	19.4	15.7	11.9
140	30.5	27.8	25.0	22.2	19.5	29.5	26.4	23.2	20.0	16.9	28.0	24.4	20.8	17.3	13.7
160	30.7	28.0	25.4	22.7	20.0	30.0	27.0	24.0	21.1	18.1	28.8	25.4	22.0	18.7	15.3
180	-----	-----	-----	-----	-----	30.3	27.4	24.6	21.8	18.9	29.4	26.2	23.0	19.8	16.6
200	-----	-----	-----	-----	-----	30.5	27.7	25.0	22.2	19.4	29.8	26.8	23.7	20.6	17.6
220	-----	-----	-----	-----	-----	30.6	27.9	25.2	22.5	19.8	30.1	27.2	24.2	21.3	18.4
240	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	30.3	27.5	24.6	21.8	19.0
260	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	30.5	27.7	25.0	22.2	19.4
280	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	30.6	27.9	25.2	22.4	19.7
300	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	30.7	28.0	25.3	22.6	19.9

Main-stand age (years)	Site index 4					Site index 5				
	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*	Ratio group *a*	Ratio group *b*	Ratio group *c*	Ratio group *d*	Ratio group *e*
20	6.5	4.8	3.1	1.4	-----	5.8	4.3	2.7	1.2	-----
40	11.3	8.6	5.9	3.2	-----	9.6	7.2	4.9	2.5	-----
60	16.2	12.7	9.2	5.7	2.2	13.7	10.6	7.4	4.3	1.2
80	20.4	16.4	12.4	8.5	4.5	17.7	14.0	10.2	6.5	2.8
100	23.4	19.3	15.2	11.1	7.0	21.1	17.0	13.0	9.0	4.9
120	25.2	21.2	17.2	13.2	9.2	23.4	19.3	15.2	11.1	7.0
140	26.5	22.6	18.8	15.0	11.1	25.0	21.0	17.0	12.9	8.9
160	27.5	23.8	20.2	16.5	12.8	26.2	22.3	18.4	14.5	10.6
180	28.3	24.8	21.3	17.8	14.3	27.1	23.4	19.6	15.8	12.1
200	28.9	25.6	22.2	18.8	15.5	27.8	24.2	20.6	17.0	13.4
220	29.3	26.1	22.9	19.7	16.5	28.4	24.9	21.4	18.0	14.5
240	29.7	26.6	23.5	20.4	17.3	28.9	25.6	22.2	18.8	15.5
260	29.9	26.9	24.0	21.0	18.0	29.3	26.1	22.8	19.6	16.4
280	30.1	27.2	24.3	21.4	18.5	29.6	26.5	23.4	20.2	17.1
300	30.3	27.5	24.6	21.8	19.0	29.8	26.8	23.8	20.7	17.7

[1] Trees 5 inches and more in d. b. h., to a 4-inch top inside bark; cull trees excluded.

Table 5, site index 1, 42 years, places the ratio of 55.0 in ratio group *a* (highest). Twenty-year ratio increase in this group is, by interpolation, 31.1, making the board-foot square-foot ratio 20 years hence 55.0+31.1, or 86.1.

Volume per acre in board feet 20 years hence is thus 173.0×86.1, or 14,895 board feet; and growth per acre for the period, 14,895—8,250, or 6,645 board feet. This means that a growth of 332 board feet per acre per year (including woods and mill cull) may be expected for the next 20 years.

In this example it was assumed that the stand belong entirely to a single size class. Where different sizes are represented on an area, a separate growth estimate should properly be made for each.

Take as a second example a stand in the eastern Upper Peninsula of Michigan, similar to the first in area and in cruise requirements. In this case, however, the timber falls in size class 3, and reference to table 2 shows that at least 200 borings are required, or 4 borings per plot. A cruise is run, and these data obtained:

(1) Average volume per acre: 10,232 board feet (Scribner).
(2) Average basal area per acre: 95.0 square feet.
(3) Board-foot square-foot ratio: 107.7.
(4) Average main-stand diameter: 17.0 inches.
(5) Average main-stand age: 276 years.

Table 3 puts this stand in site index 5, or poor. Table 4, site index 5, 276 years, puts 95.0 square feet in density index 4 (poorly stocked). Basal-area increase from 276 to 296 years is, by interpolation, 3.6 square feet, making basal area 20 years hence 98.6 square feet.

Table 5, site index 5, 276 years, indicates that a ratio of 107.7 falls in group *b*. Ratio increase in this group from 276 to 296 years is 3.2, meaning that the board-foot square-foot ratio to be expected 20 years hence is 110.9.

Volume per acre 20 years hence is 98.6×110.9, or 10,935 board feet Scribner. Growth for the 20-year period is 703 board feet, and growth per acre per year 35 board feet gross Scribner.

These two examples make it apparent that volume growth is controlled chiefly by the four factors: Site, age, density, and the proportion of small trees just ready to enter merchantability. The last factor is reflected in the ratio of volume to basal area.

SUMMARY

A method is presented for determining site and predicting growth in uneven-aged timber. Taking advantage of the fact that a main even-aged group of trees may be distinguished within each uneven-aged stand, the basal areas of stands are curved over the ages of these main groups, and these curves are used in conjunction with the ratio of volume to basal area in predicting future yields and growth. Site is introduced as a function of age and average diameter. This series of relationships may be expressed in the form of site and growth tables, which can then be applied to any stand of the cover type in question.

A statistical analysis of the field data obtained in the northern-hardwood cover type shows that (1) use of the three correlations—basal area with average diameter, volume basal-area ratio with average diameter, and age with average diameter—as a basis for growth prediction, is well justified; (2) significant, even-aged groups of trees are actually present in so-called "uneven-aged" stands; (3) only a small error is involved in determining the average age of these groups, this error depending upon the number of sample plots taken, and to some extent upon the number of age-sample trees bored on each plot.

RELATION OF CERTAIN PLANT CHARACTERS TO STRENGTH OF STRAW AND LODGING IN WINTER WHEAT [1]

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INTRODUCTION

Lodging of wheat is often an important factor influencing the final yield of grain in the more humid sections of the country. Hence the development of varieties resistant to lodging is an important objective in most breeding programs. However, lodging under field conditions usually occurs so irregularly that an accurate evaluation of the tendency of new strains and varieties to lodge is difficult to obtain. Because of this, numerous efforts have been made to find a satisfactory method of testing new strains with respect to this characteristic. Ordinarily, these have consisted of mechanical devices designed to measure resistance of the culm either to bending or to breaking. Other studies have been concerned with plant characters which, it seems, might be associated with resistance to lodging.

REVIEW OF LITERATURE

The literature on lodging is rather extensive and extends at least as far back as 1789, when Sir Humphrey Davy (*4*)[3] associated lodging of cereals with low silica content of the straw. Comprehensive reviews of early literature have been given by Welton and Morris (*13*), the Imperial Bureau of Plant Genetics, School of Agriculture,[4] Phillips, Davidson, and Weihe (*8*), and others. The reader is referred to these publications for results of research prior to 1931.

Brady (*1*) recently made extensive studies of external and internal characters of three oat varieties in an attempt to find some morphological or anatomical character associated with lodging. He found that thickness of the culm wall, number of vascular bundles, width of lignified tissue, and width of sclerenchyma were all closely associated with varietal differences in lodging, but that external characters such as height of plant and length and diameter of lower internodes were equally good indexes. He concluded that all characters were so subject to soil variation that their use for the isolation of strains resistant to lodging could be used only on a relative basis. Clark and Wilson (*2*) studied the relationship of lodging, tillering, and

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[2] The author expresses his appreciation to Dr. H. H. Laude, Department of Agronomy, Kansas State College of Agriculture and Applied Science; to Dr. K. S. Quisenberry, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture; and to Dr. P. C. Mangelsdorf and P. B. Dunkle, of the Texas Agricultural Experiment Station, for helpful suggestions in carrying out the experiment and preparing the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 119.

[4] IMPERIAL BUREAU OF PLANT GENETICS, SCHOOL OF AGRICULTURE. LODGING IN CEREALS—A REPORT OF RESEARCH. 8 pp. 1931. [Mimeographed.]

breaking strength of straw in varieties of spring wheat and barley. They found significant differences in breaking strength between varieties but no correlation between breaking strength and lodging in the field. A significant correlation between diameter of culm and breaking strength was found. Hall (*6*), in a study of lodging in corn, found lodging significantly correlated with the force necessary to pull the plant from the soil, but little or no relationship between the amount of lodging and ear height, length of underground stem (mesocotyl), stalk cross section, amount of disease of the stalk, size of brace roots, number of suckers, or weight of ears.

Smith (*12*) studied breaking strength of F_2 and F_3 strains from a cross of Gopher × Rainbow oats. He concludes that the use of the breaking strength of straw test as a means of distinguishing plants superior in this respect is not justified. Ramiah and Dharmalingam (*9*) found only a single-factor difference between strong- and weak-strawed varieties of rice.

Salmon (*10*) devised an instrument for measuring the strength of straw of small grains and he presented data to show that this measurement was correlated with lodging behavior in the field. This instrument has also been used by Salmon and Laude (*11, pp. 57–58*), Davis and Stanton (*3*), and Leidigh, Mangelsdorf, and Dunkle (*7*), and their data support Salmon's conclusions.

MATERIAL AND METHODS

The data obtained in this study were secured during the crop seasons of the 4-year period 1931–34 from plants of various wheat varieties grown at Texas Substation No. 6, Denton, in north-central Texas. The number of varieties varied from a minimum of 18 in 1931 to a maximum of 129 in 1934. Prior to 1934 the material for testing was obtained from the guard rows of the replicated wheat nursery plots. In 1934, a special seeding was made in single three-row, 8-foot plots. Plants for the study were taken from the center row of each plot. The rate of seeding was uniformly 8 grams per row.

Various characters were studied, including the breaking strength of straw, height of plants, length of lower internode, diameter and weight per unit length of culm, date of maturity, weight of grain from 100 heads, weight of 100 culms, and stand.

Determinations on strength of straw were made with the machine devised by Salmon and referred to above. Plants for testing were pulled or cut at the surface of the ground and cured under cover before tests and measurements were taken. Twenty determinations of 5 straws each, or a total of 100 straws, were made for each variety. The data are reported as the average number of pounds required to break five straws. Tests were made in all instances on the first upright internode above the crown of the plant.

Diameter of culm, length of culm, weight per unit length of culm, and length of the lower internode are based on 100 determinations for each variety. Each individual determination of weight per unit length of culm consisted of the weight of a 10-cm section, including the one node, between the internode used for the strength-of-straw determinations and the one immediately above. Plant height was measured in the field in 1932 and included the height of the entire plant. In 1933 and 1934 the length of the culm after the head was removed was determined. Whenever lodging occurred a record was

made consisting of estimates of the percentage of the plants permanently lodged.

Statistical treatment of the data has been confined to measures of the variability of breaking strength and the calculation of coefficients of correlation. The data on strength of straw were analyzed by the analysis of variance method as developed by Fisher (*5*). Simple correlation coefficients were determined for all possible combinations among the characters studied. Partial correlation coefficients were determined in a few instances where it seemed they might be of value. Many of the varieties are susceptible to rust, and in 1934, when it appeared that rust would probably interfere with the principal objective of the experiment, the entire planting was dusted with sulphur at frequent intervals, and rust did not develop in damaging amounts at least until late in the season. The breaking strength of the straw of rusted and nonrusted culms of certain varieties was determined, and no significant differences were observed. This, together with general observations, indicates that for the purposes of this study rust probably did not materially influence the results.

A considerable number of the varieties grown at Denton have been grown also at other experiment stations in the United States at which lodging has occurred. Data on lodging supplied by the various stations have been studied in relation to the breaking strength of the straw as determined at Denton, and the results are reported herein.

EXPERIMENTAL RESULTS

The data on strength of straw, measurements of certain morphological characters, and records of stand, date of maturity, lodging, and rust for the various varieties grown at the Denton substation in 1932, 1933, and 1934 are given in tables 1, 2, and 3. In each table the varieties are listed in the order of breaking strength of straw. Determinations of breaking strength of straw were also made in 1931, but no other data were recorded. The analysis of variance for the breaking strength of the straw is given in table 4, and the correlation coefficients for various pairs of characters are given in table 5.

TABLE 1.—*Breaking strength of straw and various agronomic characters of 65 wheat varieties and strains grown at Texas Substation No. 6, Denton, Tex., 1932*

Variety or strain	T. S. No.[1]	C. I. No.[2]	Date of first heading	Rust		Plant height	Length of lower internode	Diameter of culm	Estimated lodging	Weight required to break 5 culms
				Leaf	Stem					
				Pct.	*Pct.*	*Cm*	*Cm²*	*Mm*	*Pct.*	*Lb.*
Clarkan	20400	8858	Apr. 28	45	40	107	8.78	3.03	T[3]	6.06
Harvest Queen	15837	6199	Apr. 29	83	35	108	9.16	2.95	1	5.89
Ohio, T. N. 1047	18567	------	---do----	71	50	104	7.76	2.87	1	5.80
Kawvale	12577	8180	Apr. 27	21	18	99	8.05	2.85	1	5.25
P1066×Burbank	20425	10087	Apr. 25	63	18	97	6.80	2.66	9	5.23
Mediterranean selection	5933-36	------	Apr. 24	13	24	107	10.00	2.87	24	5.17
Fultz	20474	3416	Apr. 22	79	9	91	7.25	2.83	4	5.08
Mediterranean selection	5933-32	------	Apr. 23	26	35	102	9.03	2.63	34	4.92
Dó	5933-7	------	---do----	21	18	102	9.86	2.71	38	4.91

[1] Accession number of Texas Agricultural Experiment Station.
[2] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.
[3] T=trace.

TABLE 1.—*Breaking strength of straw and various agronomic characters of 65 wheat varieties and strains grown at Texas Substation No. 6, Denton, Tex., 1932*—Con.

Variety or strain	T. S. No.	C. I. No.	Date of first heading	Rust		Plant height	Length of lower internode	Diameter of culm	Estimated lodging	Weight required to break 5 culms
				Leaf	Stem					
				Pct.	*Pct.*	*Cm*	*Cm*	*Mm*	*Pct.*	*Lb.*
Fultzo-Mediterranean selection	20429	-------	Apr. 27	75	8	99	8.21	2.82	3	4.84
Fulcaster	7082	6471	Apr. 29	78	43	107	8.40	2.62	8	4.81
Sutton	15832	10053	May 1	24	50	102	8.25	2.54	13	4.80
Smithsonian	20431	10022	May 2	20	30	104	7.07	2.57	64	4.79
Sibley No. 81	20427	10084	Apr. 27	45	10	99	7.95	2.52	18	4.76
Early Blackhull	15838	8856	Apr. 20	83	T	89	8.63	2.59	2	4.71
Denton (average of 6 check plots)	9236	8265	Apr. 27	15	24	102	8.42	2.53	9	4.70
Kanred×Fulcaster selection	20401	-------	Apr. 26	26	15	102	9.05	2.76	3	4.69
Mediterranean selection	5933-35	-------	Apr. 23	11	21	108	9.68	2.68	36	4.64
Kanred×Marquis	20426	11374	Apr. 24	36	3	89	7.58	2.51	8	4.46
Quivira	15833	8886	Apr. 23	30	5	94	7.54	2.44	9	4.45
Mediterranean selection	5933-23	11525	Apr. 25	4	16	107	8.19	2.67	10	4.44
Do	5933-38	-------	Apr. 23	13	25	107	10.30	2.70	30	4.37
Do	3015-105-1	11587	Apr. 27	14	40	104	9.11	2.61	21	4.36
White Mediterranean	15834	10023	Apr. 23	68	41	102	9.26	2.47	10	4.36
Kanred×Marquis	20390	10090	Apr. 29	13	14	99	10.17	2.65	29	4.34
Minturki	20483	6155	May 1	82	10	107	7.75	2.45	2	4.30
Mediterranean selection	3015-63	-------	Apr. 26	6	23	99	9.57	2.50	9	4.20
Kanred×Marquis, Kansas 2640	20391	-------	Apr. 27	23	9	97	9.37	2.56	4	4.14
Kanred×Fulcaster selection	23049	-------	do	31	23	104	9.33	2.56	21	4.12
Mediterranean selection	3015-130	-------	Apr. 26	21	24	102	8.71	2.44	9	4.07
Blackhull	7172	6251	Apr. 29	80	8	99	8.73	2.38	20	4.06
Kanred×Fulcaster	20402	-------	Apr. 28	18	13	104	9.39	2.63	8	4.05
Beloglina selection	20424	8884	May 2	74	13	97	7.38	2.37	35	4.03
Kanred×Hard Federation	20418	10092	Apr. 22	48	10	86	7.77	2.50	4	4.02
Turkey selection	20376	10100	Apr. 30	88	26	91	7.39	2.34	50	4.02
Tenmarq	12578	6936	Apr. 29	44	9	99	8.13	2.48	11	3.93
Mediterranean selection	5933-20	10085	Apr. 24	15	11	99	9.13	2.30	13	3.92
Do	5933-34	11526	do	24	11	99	9.68	2.32	9	3.90
Red May selection	7250-1	-------	Apr. 27	30	25	89	8.94	2.44	9	3.90
Mediterranean selection	3015-81	10086	Apr. 26	33	16	97	7.82	2.29	33	3.90
Kanred selection	20415	10099	May 1	76	10	99	8.89	2.48	28	3.90
Cheyenne	18566	8885	do	78	10	94	8.14	2.51	6	3.87
Oro	20389	8220	do	75	8	99	8.81	2.53	6	3.85
Kanred×Hard Federation	20416	11373	Apr. 21	51	8	86	7.68	2.47	4	3.79
Tenmarq selection	20421	10089	Apr. 29	35	8	99	8.58	2.56	11	3.77
Turkey selection	20370	10094	May 1	96	8	89	7.24	2.26	4	3.70
Nebraska No. 60	15835	6250	do	88	14	97	8.00	2.36	19	3.66
Kharkof	16830	1442	do	81	11	97	7.12	2.26	10	3.64
Mediterranean selection	3015-72	11567	Apr. 27	48	23	99	8.82	2.29	39	3.59
Kanred×Hard Federation	20419	10093	Apr. 19	46	6	84	7.78	2.39	0	3.58
Fulhard	20420	8257	Apr. 26	80	11	97	9.43	2.35	5	3.54
Turkey selection	20375	10098	Apr. 28	94	9	91	7.84	2.23	4	3.53
Kanred×Hard Federation	20417	10091	Apr. 19	38	4	84	8.35	2.47	0	3.51
Nebraska No. 28	15836	5147	Apr. 15	85	0	74	8.37	2.31	0	3.50
Turkey selection	20371	10095	Apr. 30	94	10	86	7.20	2.23	6	3.49
Do	20368	10083	Apr. 29	75	8	97	8.32	2.27	39	3.48
Minturki×Beloglina-Buffum	20422	10088	May 2	81	9	102	8.43	2.26	24	3.48
Kanred×Minturki	20423	10012	do	75	9	99	7.61	2.23	29	3.45
Canadian hybrid	20428	-------	Apr. 20	88	10	91	8.17	2.27	13	3.42
Kanred	11763	5146	May 1	76	8	91	9.59	2.35	35	3.40
Turkey selection	20373	10097	Apr. 29	94	8	89	6.41	2.09	10	3.39
Do	20374	10016	Apr. 28	96	9	91	7.97	2.19	4	3.39
Do	20377	11375	Apr. 29	91	16	94	7.83	2.17	15	3.36
Do	20372	10096	do	96	9	89	6.85	2.17	4	3.30
Do	20369	10015	Apr. 30	94	6	91	6.50	2.15	6	3.26

TABLE 2.—*Breaking strength of straw and various agronomic characters of 44 wheat varieties and strains grown at Texas Substation No. 6, Denton, Tex., 1933*

Variety or strain	T. S. No.	C. I. No.	Date of first heading	Rust		Plant height	Length of lower internode	Diameter of culm	Weight of grain from 100 heads	Weight of 100 culms	Weight of 100 10-cm culm sections	Estimated lodging	Weight required to break 5 culms (average of 20 tests)
				Leaf	Stem								
				Pct.	*Pct.*	*Cm*	*Cm*	*Mm*	*G*	*G*	*G*	*Pct.*	*Lb.*
P1066 × Burbank	20425	10087	May 6	48	5	80	10.1	3.4	101	124	15.1	3	7.66
Clarkan	20400	8858	May 8	45	18	84	11.2	3.3	77	114	12.6	1	6.80
Harvest Queen	15837	6199	do	78	15	84	11.9	3.2	62	109	11.8	0	6.74
Mediterranean selection	3015–105–1	11587	do	8	49	79	9.5	3.1	71	104	12.3	1	6.30
Do	5933–23	11525	May 7	1	24	80	9.3	3.0	73	96	11.3	3	5.96
Fulhard	20420	8257	May 6	51	24	83	10.1	2.8	64	97	11.2	2	5.88
Sibley No. 81	20427	10084	May 9	48	8	71	9.7	2.7	59	82	10.5	1	5.82
Kanred × Kawvale	20409	------	May 10	5	4	78	10.5	3.0	66	93	11.6	3	5.76
Nebraska No. 28	15836	5147	Apr. 18	83	0	63	8.3	2.7	45	69	10.3	0	5.71
(Kanred × Fulcaster) × (Kanred × Hard Federation)	20405	------	May 2	11	4	82	11.4	2.9	84	91	10.6	19	5.69
Fulcaster	7082	6471	May 8	60	25	78	9.7	2.8	62	89	10.5	3	5.66
Mediterranean	3015–63	------	do	3	30	79	10.9	2.8	67	85	10.0	3	5.63
Denton × Kanred	------	------	do	15	16	87	12.0	2.9	64	93	9.3	18	5.50
Mediterranean selection	3015–72	11567	May 7	19	34	83	9.4	2.6	53	91	10.9	3	5.46
Kawvale	12577	8180	May 8	7	11	81	11.1	2.9	66	88	10.2	4	5.43
Sutton	15832	10053	do	44	18	76	10.0	2.8	55	89	9.9	3	5.38
White Mediterranean	15834	10023	May 1	85	11	78	11.2	2.7	67	81	10.0	3	5.28
Kanred × Hard Federation	20416	11373	May 2	45	23	83	10.7	2.8	74	83	10.3	18	5.26
Denton	9236	8265	May 9	12	23	85	10.2	2.7	60	86	10.0	4	5.24
Kanred × Fulcaster	20401	------	May 8	14	3	79	10.8	2.8	56	81	9.8	0	5.16
Mediterranean selection	5933–20	10085	May 5	13	13	74	9.1	2.4	55	76	9.7	13	5.09
Do	3015–81	10086	May 9	13	26	73	8.6	2.6	50	71	9.3	4	4.91
Do	5933–34	11526	May 5	14	1	83	10.3	2.5	50	74	9.2	5	4.86
Quivira	15833	8886	May 1	24	1	87	11.9	2.9	67	90	9.2	4	4.86
Tenmarq × Kawvale	20406	------	May 6	14	19	79	10.3	2.7	58	78	9.2	3	4.81
Early Blackhull	15838	8856	Apr. 27	75	1	85	10.0	2.7	59	80	9.5	4	4.71
Kanred × Kawvale	20408	------	May 9	4	0	87	12.2	2.7	47	81	7.8	0	4.62
Blackhull	7172	6251	May 8	85	1	83	10.3	2.6	46	75	8.3	6	4.58
Texas selection	20399	------	May 9	4	3	83	11.8	2.9	67	85	9.0	8	4.57
Beloglina selection	20424	8884	May 13	74	5	73	11.1	2.7	52	73	8.7	6	4.52
Turkey selection	20370	10094	May 10	95	0	65	9.4	2.6	36	58	8.3	0	4.51
Minturki	20483	6155	May 12	60	65	75	11.2	2.6	39	71	8.3	0	4.47
Denton selection	20350	------	May 10	39	9	75	11.4	2.8	54	74	9.8	4	4.38
Red May selection	7250–1	------	May 3	35	19	72	9.2	2.5	50	68	8.2	4	4.38
Kanred	11763	5146	May 10	80	1	80	11.5	2.7	50	72	7.6	4	4.28
Tenmarq	12578	6936	May 7	40	1	81	11.9	2.7	61	74	7.9	3	4.26
Oro	20389	8220	May 10	76	3	81	12.0	2.5	43	67	7.0	0	4.19
Iobred	20430	6934	do	58	10	68	9.6	2.6	39	63	8.4	0	4.13
Kanred selection	20415	10099	do	78	0	63	9.4	2.5	38	52	8.1	0	4.13
Nebraska No. 60	15835	6250	May 13	88	8	74	10.6	2.6	34	64	7.8	0	4.12
Cheyenne	18566	8885	May 10	83	1	78	10.3	2.6	40	61	7.5	0	4.10
Webster × Malakof	20410	------	do	25	6	78	10.6	2.6	47	64	6.7	14	4.04
Kharkof	16830	1442	May 12	83	9	76	10.9	2.6	34	64	7.4	6	4.03
Turkey selection	20377	11375	May 11	95	0	58	8.3	2.4	27	33	7.0	0	3.84

TABLE 3.—*Breaking strength of straw and various agronomic characters of 129 wheat varieties and strains grown at Denton, Tex., 1934*

Variety or strain	T. S. No.	C. I. No.	Date of first heading	Rust		Plant height	Culms per 8-foot row	Length of lower internode	Diameter of culm	Weight of grain from 100 heads	Weight of 100 culms	Weight of 100 10-cm culm sections	Estimated lodging	Weight required to break 5 culms (average of 20 tests)
				Leaf	Stem									
				Pct.	*Pct.*	*Cm*	*No.*	*Cm*	*Mm*	*G*	*G*	*G*	*Pct.*	*Lb*
Nittany		6962	May 2	40	5	88	194	10.1	3.36	69	119	12.9		7.52
Red Chief		3392	May 5	75	5	99	198	11.1	3.42	59	112	11.8		7.06
Powerclub		8276	do	65	10	100	284	12.6	3.36	72	118	10.8		6.71
Clarkan	20400	8858	Apr. 30	76	5	101	299	11.0	3.12	60	105	11.4	0	6.62
Longberry No. 1		5823	May 7	45	20	97	183	11.0	3.36	59	103	11.4		6.60
Coppei		4238	May 5	75	5	82	263	11.3	3.38	69	97	10.9		6.49
Rupert		5920	May 7	60	15	85	168	8.4	3.20	59	101	12.4		6.43
Prosperity		5380	May 12	98	45	74	116	9.3	3.24	45	87	11.6		6.35
Red Wave		3500	May 5	75	25	98	220	9.7	3.20	60	103	11.3		6.35
Arco		8246	Apr. 26	98	10	93	289	13.6	3.96	65	114	11.8		6.29
Fultzo-Mediterranean		4811	Apr. 30	95	10	81	201	10.0	3.10	54	85	10.3		6.20
Rochester		5693	May 5	95	5	89	220	10.8	3.16	45	98	10.3		6.20
Walker		6445	Apr. 27	85	0	100	235	10.6	2.96	48	97	10.4		6.17
China		180	May 7	95	20	89	276	10.8	3.20	44	94	11.4		6.16
Russian		5737	May 2	75	10	106	314	12.3	3.00	58	103	10.4	15	6.16
Dreadnaught	23048		Apr. 27	15	0	90	313	10.5	3.32	65	94	11.1		6.15
Purkof		8381	May 5	85	5	97	338	10.8	3.12	60	98	10.9		6.07
Democrat		3384	do	65	15	92	232	11.5	3.12	50	87	9.6		6.05
Rural New Yorker No. 6		5921	May 7	50	10	83	150	11.2	3.38	53	86	10.3		5.99
Goldcoin		4156	May 4	45	10	97	278	10.3	3.22	46	94	9.9		5.97
Genro		11535	May 7	85	30	72	222	8.4	3.30	56	85	10.5		5.96
Imperial Amber		5338	May 5	60	10	93	190	10.1	3.20	50	94	11.3		5.96
Nabob		8869	May 3	85	5	95	222	12.0	3.06	48	94	10.6		5.91
Jones Fife		4466	do	98	10	88	246	12.3	3.42	44	90	10.8		5.80
Diehl-Mediterranean		1395	May 5	50	5	100	200	10.9	2.92	54	89	9.9		5.80
Greeson		6320	Apr. 30	85	T	86	250	10.2	3.00	59	87	9.9		5.73
Eaton		4682	May 17	100	20	69	184	9.7	3.32	28	88	11.5		5.69
P1066×Burbank	20425	10087	Apr. 30	50	5	86	260	9.7	3.10	61	84	10.3	11	5.67
Denton×Kanred	23051		May 3	50	5	104	350	11.5	3.12	61	100	10.0	56	5.66
V. P. I. 131		10047	Apr. 30	85	5	89	216	10.8	2.96	51	82	10.3		5.65
Goens		4857	do	75	T	91	282	9.0	2.90	49	71	10.3		5.58
Leap		4823	Apr. 23	75	0	85	220	10.6	3.20	57	89	10.3		5.55
Honor		6161	Apr. 30	95	10	99	327	9.8	3.10	49	95	10.5		5.52
Triplet		5408	May 5	98	5	99	252	11.1	3.42	59	112	11.8		5.52
Dawson		3342	Apr. 30	98	5	95	330	8.3	3.14	49	78	10.3		5.50
Silvercoin		6013	May 5	45	10	90	268	12.3	3.28	45	91	9.3		5.49
Red May		5336	May 2	95	5	79	170	7.6	3.06	38	73	10.2		5.43
Gypsy		3436	May 7	85	5	80	174	9.2	2.98	49	72	10.1		5.42
Genesee Giant		1744	Apr. 26	85	10	74	163	9.9	3.14	48	69	9.5		5.36
Quivira	15833	8886	do	65	T	88	363	10.1	2.90	59	75	9.3	5	5.30
Denton	9236	8265	May 1	34	5	95	335	10.9	2.78	48	83	9.1	8	5.29
Harvest Queen	15837	6199	May 3	85	10	96	290	12.9	3.08	40	81	8.5	1	5.27
Russian Red		5928	May 5	95	25	90	232	10.7	3.18	55	90	10.1		5.26
Sol		6009	May 16	65	25	70	178	9.7	3.46	40	89	11.4		5.24
Red Clawson		3393	May 2	95	5	86	222	10.7	3.22	50	79	10.1		5.24
Mediterranean selection	5933–23	11523	Apr. 30	5	10	97	290	12.1	2.76	45	80	8.9	4	5.17
Oakley		6301	Apr. 23	75	5	72	192	8.1	2.70	41	69	8.5		5.16
Texas selection	20399		May 6	5	10	81	242	11.3	2.88	54	68	8.7	50	5.14
Illini Chief		5406	Apr. 30	95	T	85	246	11.0	2.96	46	77	8.9		5.12
White Winter		5219	May 17	99	20	77	216	10.1	3.00	37	87	9.2		5.11
Mediterranean	3015–63		Apr. 30	50	5	100	306	12.6	2.78	47	84	8.3	5	5.08
Portage		5654	May 5	50	10	93	227	11.4	3.20	50	89	9.1		5.06
Forward		6691	May 2	55	5	91	276	13.3	2.98	54	82	9.1		5.04
Fishhead × Velvet Chaff 51	23047		Apr. 17	65	0	70	154	9.6	3.16	73	73	9.6		5.04
Odessa		4475	May 5	75	20	86	206	9.9	3.00	48	88	10.5		5.02
Mealy		3358	Apr. 30	99	10	91	293	10.5	2.94	51	84	9.6		5.02
Berkeley Rock		8272	May 7	75	5	86	242	8.8	2.88	40	75	9.3		5.02
Golden		10063	May 6	45	20	81	252	10.8	3.30	45	80	10.1		5.02
Currell		3326	Apr. 30	70	5	88	233	10.5	2.98	49	81	9.5		5.00
Hybrid 128		4512	May 9	99	20	80	232	7.5	2.96	38	76	9.1		4.98
Valley		5923	May 7	85	15	83	209	11.2	2.80	39	74	9.6		4.97
Rice		5734	Apr. 18	85	0	79	254	10.6	2.84	50	74	9.4		4.95
Tenmarq × Kawvale	20406		May 1	20	0	90	354	11.2	2.90	52	74	9.1	8	4.94
Mosida		6688	May 2	99	5	79	240	9.3	2.76	45	71	8.9		4.94

TABLE 3.—*Breaking strength of straw and various agronomic characters of 129 wheat varieties and strains grown at Denton, Tex., 1934*—Continued

Variety or strain	T. S. No.	C. I. No.	Date of first heading	Rust		Plant height	Culms per 8-foot row	Length of lower internode	Diameter of culm	Weight of grain from 100 heads	Weight of 100 culms	Weight of 100 10-cm culm sections	Estimated lodging	Weight required to break 5 culms (average of 20 tests)
				Leaf	Stem									
				Pct.	*Pct.*	*Cm*	*No.*	*Cm*	*Mm*	*G*	*G*	*G*	*Pct.*	*Lb.*
Mediterranean selection	3015–81	10086	Apr. 27	65	5	90	348	9.6	2.66	40	71	8.6	5	4.93
Sutton	15832	10053	May 3	30	10	90	314	11.0	2.80	44	79	8.5	5	4.93
Fishhead × Velvet Chaff	23045	-----	Apr. 19	65	0	78	182	9.0	3.18	77	90	10.8	----	4.92
Kanred × Fulcaster selection	20401	-----	May 5	10	0	88	356	11.1	2.94	53	71	8.4	3	4.86
Shepherd	----------	6163	Apr. 30	95	T	81	211	9.2	2.88	46	76	9.3	----	4.84
Minturki	----------	6155	May 6	75	0	81	238	9.3	2.34	36	62	8.4	----	4.82
Kinney	----------	5189	May 3	65	10	81	356	9.6	2.76	42	69	8.3	----	4.82
Michikof	----------	6990	May 5	99	15	89	256	10.2	2.70	41	75	8.3	----	4.81
Mediterranean selection	5933–20	10085	Apr. 26	40	10	83	330	9.8	2.46	38	62	7.3	18	4.81
Redhart	----------	8898	Apr. 23	85	0	84	270	9.2	2.96	51	76	8.9	----	4.80
Red Indian	----------	8382	May 6	55	5	81	228	10.4	2.36	47	67	8.8	----	4.80
Mammoth Red	----------	2008	June 7	85	15	83	166	9.5	2.34	42	74	9.1	----	4.79
Kofod	----------	4337	May 2	75	10	91	256	11.3	2.90	45	78	8.3	----	4.78
Wheedling	----------	4846	May 5	95	5	80	202	9.2	2.98	42	79	10.1	----	4.75
Fulhio	----------	6999	May 2	85	T	90	206	10.9	2.94	46	79	8.9	----	4.74
Gladden	----------	5644	---do----	65	25	74	206	10.1	2.92	43	65	8.4	----	4.74
White Mediterranean	15834	10023	Apr. 25	99	10	81	284	10.9	2.68	49	69	8.1	44	4.74
Rudy	----------	4873	May 4	85	5	103	271	11.3	3.00	52	88	9.1	----	4.73
Gold Drop	----------	6316	Apr. 18	85	5	76	236	10.2	2.64	47	69	8.1	----	4.73
Gasta	----------	11398	Apr. 20	65	0	78	288	9.4	2.54	45	67	8.4	----	4.71
Kanred × Hard Federation	20416	11373	Apr. 27	75	5	80	346	10.2	2.84	49	65	8.3	3	4.71
Sibley No. 81	20427	10084	May 5	65	5	94	292	10.9	2.80	47	73	8.5	26	4.71
Nigger	----------	5366	---do----	85	5	78	206	9.7	2.94	40	68	8.7	----	4.68
Red Rock	----------	5597	May 3	75	T	87	292	9.3	2.80	42	65	8.5	----	4.65
Mediterranean selection	5933–34	11526	Apr. 27	40	5	82	362	10.8	2.36	37	58	7.1	16	4.60
Poole	----------	3488	May 5	95	10	90	214	11.7	3.02	39	74	8.2	----	4.56
Kanred selection	20415	10099	---do----	50	5	81	288	11.4	2.72	50	60	7.5	33	4.54
Cooperatorka	----------	8861	May 2	99	0	81	321	9.8	2.62	35	62	8.1	----	4.49
Minhardi	----------	5149	Apr. 18	75	0	82	230	9.9	2.96	48	75	9.3	----	4.48
Fishhead × Velvet Chaff	23046	-----	Apr. 17	65	0	73	204	10.3	2.92	68	65	8.9	----	4.47
Kawvale	12577	8180	May 3	15	0	94	370	11.6	2.88	48	76	8.4	1	4.41
(Kanred × Fulcaster) × (Kanred × Hard Federation)	20405	-----	Apr. 25	25	5	87	359	12.7	2.78	60	69	7.3	28	4.38
Early Blackhull	15838	8856	Apr. 23	85	2	77	239	9.2	2.50	43	54	7.5	15	4.37
Fultz	20474	3416	Apr. 20	75	0	78	274	11.3	2.80	43	68	8.1	61	4.33
Tenmarq	12578	6936	May 3	70	T	88	333	10.7	2.80	49	69	8.1	21	4.33
Trumbull	----------	5657	May 5	95	5	74	197	10.7	2.72	40	65	7.6	----	4.32
Fulcaster	7082	6471	May 3	60	10	89	284	11.2	2.52	42	64	6.9	6	4.32
Flint	----------	6307	Apr. 20	75	0	85	315	11.4	2.84	48	74	7.8	0	4.31
Purplestraw	----------	1915	Apr. 19	95	0	72	322	9.4	2.38	42	56	7.7	----	4.28
Ridit	----------	6703	May 3	99	5	90	292	11.4	2.60	39	68	7.0	----	4.27
Mediterranean selection	3015–105–1	11587	Apr. 29	20	5	95	344	10.6	2.80	48	73	7.9	18	4.26
Zimmerman	----------	2907	Apr. 18	65	0	79	244	9.7	2.78	44	69	8.3	----	4.23
Fulhard	20420	8257	May 5	99	0	79	282	9.4	2.68	37	57	7.8	4	4.22
Webster × Malakof	20410	-----	---do----	40	0	102	295	13.5	2.88	53	74	7.8	78	4.20
Ashkof	----------	6680	May 8	65	5	77	232	11.0	2.72	33	57	7.5	----	4.12
Beloglina selection	20424	8884	May 9	60	0	77	312	11.1	2.52	45	56	6.4	31	4.11
White Odessa	----------	4655	May 7	95	10	78	272	10.4	2.62	30	60	7.3	----	3.99
Mediterranean selection	3015–72	11567	Apr. 29	45	5	91	334	11.3	2.22	40	63	7.3	10	3.97
Windsor	----------	5915	May 2	85	30	61	228	7.0	2.74	31	51	7.6	----	3.95
Iobred	20430	6934	May 7	99	10	80	243	11.7	2.66	30	57	7.1	----	3.94
Blackhull	7172	6251	May 2	85	5	84	352	9.8	2.52	34	59	6.9	26	3.88
Cheyenne	18566	8885	May 6	85	T	71	310	10.3	2.68	36	49	7.1	8	3.86
Kanred	11763	5146	---do----	60	0	89	354	12.1	2.62	43	62	6.3	73	3.82
Alton	----------	1438	May 7	99	10	85	308	11.8	2.60	39	61	6.6	13	3.79
Ashland	----------	6692	May 5	95	T	78	266	10.9	2.60	32	55	7.1	----	3.78
Prohibition	----------	4068	May 8	85	T	65	283	14.8	3.22	36	66	9.3	----	3.77

TABLE 3.—*Breaking strength of straw and various agronomic characters of 129 wheat varieties and strains grown at Denton, Tex., 1934*—Continued

Variety or strain	T. S. No.	C. I. No.	Date of first heading	Rust		Plant height	Culms per 8-foot row	Length of lower internode	Diameter of culm	Weight of grain from 100 heads	Weight of 100 culms	Weight of 100 10-cm culm sections	Estimated lodging	Weight required to break 5 culms (average of 20 tests)
				Leaf	Stem									
				Pct.	*Pct.*	*Cm*	*No.*	*Cm*	*Mm*	*G*	*G*	*G*	*Pct.*	*Lb.*
Oro	20389	8220	May 6	85	T	71	276	10.3	2.48	37	47	6.4	----	3.76
Nebraska No. 60	15835	6250	May 7	98	T	73	244	10.0	2.52	36	49	6.2	16	3.69
Sherman	----------	4430	May 8	98	5	70	276	11.2	2.48	32	46	6.0	----	3.57
Wisconsin Pedigree No. 2	----------	6683	May 7	98	T	78	272	9.2	2.54	27	51	6.5	----	3.51
Turkey	----------	1558	May 8	85	T	67	332	8.5	2.38	34	40	6.1	----	3.48
Nebraska No. 28	15836	5147	Apr. 15	85	0	66	297	9.8	2.44	39	48	6.8	0	3.48
Huston	----------	5208	Apr. 23	99	5	66	259	8.9	2.36	27	40	6.3	----	3.36
Early Kanred	----------	8261	Apr. 14	85	0	67	298	9.3	2.20	38	42	6.6	----	3.30
Kharkof	16830	1442	May 6	85	0	70	292	11.4	2.36	32	42	5.1	26	3.21

TABLE 4.—*Analysis of variance of strength of straw determinations of winter wheat varieties grown at Denton, Tex., 1932, 1933, and 1934*

Variance	Degrees of freedom	Sum of squares	Mean square	Standard error of the mean of any variety	Least significant difference between any two varieties
1932				*Pounds*	*Pounds*
Total	1,299	892.612	0,687		
Between varieties	64	571.085	8.923	0.114	0.316
Within varieties	1,235	321.527	.260		
1933					
Total	879	1,068.087	1.215		
Between varieties	43	617.146	14.352	.164	.455
Within varieties	836	450.941	.539		
1934					
Total	2,579	3,106.018	1.204		
Between varieties	128	1,886.830	14.741	.158	.438
Within varieties	2,451	1,219.188	.497		

It will be noted that the varieties differed markedly with respect to most of the characters. Thus, in the seasons when these relations were studied the following differences existed: In 1933 and 1934 (tables 2 and 3) the highest value for the weight of grain per 100 heads exceeded that of the lowest by 274 and 185 percent, respectively; the highest weight of 100 culms, by 276 and 198 percent; and the greatest weight per unit length of culm, by 125 and 153 percent. During 1932–34 (tables 1–3) the highest breaking strength of straw exceeded the lowest by 86, 99, and 134 percent, respectively; in length of lower internode the highest values exceeded the lowest by 61, 47, and 94 percent; and the greatest diameter of culm at base exceeded the lowest by 45, 42, and 80 percent. The range in date of maturity was 17 days in 1932, 25 days in 1933, and 33 days in 1934. Table 4 shows that the range in breaking strength of straw was many times greater than the standard error. Statistical analysis of the other determinations was not attempted.

TABLE 5.—*Correlation coefficients for each pair of characters studied in winter wheat varieties grown at Denton, Tex., in 1932, 1933, and 1934*

Year and characters	Breaking strength of straw	Height of plant	Lower internode (length)	Culm at base (diameter)	Date of maturity	Grain from 100 heads (weight)	100 culms (weight)	Unit length of culm at base (weight)	Stand (culms per 8-foot row)
1932 [1]									
Lodging in the field	−0.046	0.286	0.159	−0.063	0.259	------	------	------	------
Breaking strength of straw	------	.613	.253	.861	−.112	------	------	------	------
Height of plant	------	------	.479	.682	.322	------	------	------	------
Length of lower internode	------	------	------	.501	.216	------	------	------	------
Diameter of culm at base	------	------	------	------	−.056	------	------	------	------
1933 [2]									
Lodging in the field	.025	.327	.208	.023	−.209	0.347	0.126	0.003	------
Breaking strength of straw	------	.348	.004	.903	−.355	.788	.895	.956	------
Height of plant	------	------	.664	.382	.086	.563	.662	.294	------
Length of lower internode	------	------	------	.256	.267	.250	.292	.078	------
Diameter of culm at base	------	------	------	------	.023	.896	.964	.887	------
Date of maturity	------	------	------	------	------	−.256	−.229	−.353	------
Weight of grain from 100 heads	------	------	------	------	------	------	.871	.814	------
Weight of 100 culms	------	------	------	------	------	------	------	.869	------
1934 [3]									
Lodging in the field	−.196	.056	.351	−.001	.178	.158	−.575	−.210	−0.039
Breaking strength of straw	------	.542	−.411	.789	.152	.544	.901	.911	−.330
Height of plant	------	------	.435	.423	.129	.142	.672	.431	.296
Length of lower internode	------	------	------	.249	.114	−.183	.229	−.010	.342
Diameter of culm at base	------	------	------	------	.238	.324	.811	.868	−.396
Date of maturity	------	------	------	------	------	−.478	.153	.143	−.213
Weight of grain from 100 heads	------	------	------	------	------	------	.648	.524	−.433
Weight of 100 culms	------	------	------	------	------	------	------	.893	−.317
Weight per unit length of culm at base	------	------	------	------	------	------	------	------	−.518

[1] Least significant value of $r=0.250$; least highly significant value of $r=0.325$.
[2] Least significant value of $r=0.288$; least highly significant value of $r=0.372$.
[3] Least significant value of $r=0.195$; least highly significant value of $r=0.254$, except for lodging, in which case the coefficient was calculated for 40 varieties only. For these cases the least significant value of $r=0.304$.

The chief interest here is with respect to lodging, since the tendency to lodge is the factor it is desirable to measure. All but 3 of the 65 varieties and strains grown in 1932 showed some tendency to lodge, the amount of lodging varying from a trace to 64 percent. In 1933, 32 of the 44 varieties and strains showed a tendency to lodge, but the amount of lodging was small, varying from 1 to 19 percent. No lodging occurred in the planting made for strength of straw studies in 1934. However, 39 of the varieties and strains were also included in the replicated yield nursery, and it was from these plantings that lodging data were taken in that year. In this case all but three varieties showed a tendency to lodge, the amount of lodging varying from 1 to 73 percent. It must be admitted that the data on lodging are not so satisfactory as might be desired, but, on the other hand, they are probably as good as can reasonably be expected, if due consideration is given to seasonal variation and the numerous factors known to affect lodging. On the whole, it would seem that the range in lodging and of other characters studied is such as to make a study of the relation of these characters and tendency to lodge at least fairly satisfactory. It now remains to be determined whether lodging is correlated with any of the plant characters that were studied, and if so to what extent.

LODGING AND BREAKING STRENGTH OF STRAW

Lodging was not significantly correlated with breaking strength of straw in any season (table 5). It was significantly and positively correlated with height of plant in 1932 and 1933, with length of the lower internode in 1934, with date of maturity in 1932, and with weight of grain per 100 heads in 1933, and was significantly and negatively correlated with weight per 100 culms in 1934. All other coefficients involving lodging failed to reach the level of significance. The coefficients for lodging and length of the lower internode were positive in each of the three seasons, and in one of them statistically significant. When combined by Fisher's method (*5*) a coefficient of 0.226 was obtained, which was highly significant.

Because of the influence of these various factors on lodging, it was considered desirable to determine whether, by holding these constant by means of partial correlation coefficients, the relation between breaking strength and lodging might be made more apparent. It was found that this procedure made no material changes in the coefficients for 1932 and 1933, and hence the partial correlation coefficients for those years are not given in detail. The coefficients for 1934 are as follows:

Characters held constant	*Partial coefficient*
Height of plant	—0. 270
Length of lower internode	—. 061
Date of maturity	—. 229
Height of plant and length of lower internode	—. 011
Height of plant and date of maturity	—. 291
Length of lower internode and date of maturity	—. 110

It will be noted that, whereas the simple correlation coefficient between breaking strength and lodging was —0.196, the partial coefficient with height of plant constant was —0.270. Similarly, this coefficient was increased to —0.229 by holding date of maturity constant and to —0.291 by holding both height of plant and date of maturity constant. None of these values, however, reaches the level of statistical significance (0.304). On the other hand, holding length of internode constant decreased the coefficient.

One of the serious difficulties in evaluating the tendency of varieties to lodge is the very great variation in lodging from year to year and from place to place. It would seem, therefore, that an average for a number of years might be useful. Eighteen varieties were grown at Denton in each of the 4 years, 1931–34, and 36 were grown in each of the 2 years, 1933–34. The correlation coefficient for average lodging and for average breaking strength was —0.626 for the 18 varieties for the 4-year period, and —0.387 for the 36 varieties for the 2-year period, both coefficients being statistically significant.

Additional information is afforded by a number of varieties grown at different experiment stations, mostly midwestern, the lodging data for which have been supplied by the agricultural experiment stations of the respective States. Table 6 gives these data, together with lodging, breaking strength of straw, and the weight per unit length of culm for the same varieties grown at Denton. Since all varieties were not grown each year, the average lodging of each variety is expressed as a percentage of the average lodging of the single variety, Blackhull, for the same years. This places all varieties on a comparable basis so far as this is possible. The correlation coefficient for lodging and breaking strength is —0.422, which is highly significant.

TABLE 6.—*Comparison of field lodging of wheat varieties grown at selected experiment stations with breaking strength and weight per unit length of culm at Denton, Tex.*

Variety	T. S. No.	C. I. No.	Percentage of lodging during indicated year at—																								Average	Average expressed as percentage of lodging in Blackhull (same experiments)	Average percentage of lodging in Blackhull (same experiments)	Crop years compared	Denton, Tex., 1934	
			Denton, Tex.				Manhattan, Kans.								Urbana, Ill.		New Brunswick, N. J.		Bozeman, Mont.				Lincoln, Nebr.		Woodward, Okla.	Fort Collins, Colo.							
																	Nursery	Field plot															
			1931	1932	1933	1934	1927	1928	1929	1930	1931	1932	1934	1935	1924	1931	1928	1928	1931	1933	1934	1935	1932	1935	1935	1934	1935					Breaking strength of culm	Weight per unit of culm
			Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*No.*	*Lb.*	*G*
Nittany		6962									0	5												1			2.0	12.3	16.3	3	7.52	12.9
Clarkan	20400	8858	1	0	1	0			35	45	55	18	0	3										15	3		14.7	43.5	33.8	12	6.62	11.4
Dreadnaught	23048									0		0		0													.0	.0	53.0	3	6.15	11.1
Purkof		8381								25	52	0	0	15		10	5	27						0			14.9	30.9	48.2	9	6.07	10.9
Democrat		3384									15	8						20									14.3	25.9	55.3	3	6.05	9.6
P1066 × Burbank	20425	10087		9	3	11					50	15	10	4						0			0	0	5	49	5	12.4	40.8	30.4	13	5.67	10.3
Leap		4823									7		0	0			5	42						0			9.0	25.7	35.0	6	5.58	10.3
Red May		5336	2	9	4	5											30										10.0	51.0	19.6	5	5.43	10.2
Quivira	15833	8886	2	9	4	5	46	37	67	83	85	22	3	7					5	0	7	15		5	11		22.9	58.4	39.2	18	5.30	9.3
Denton	9236	8265	4	9	4	8							10	10											9		7.7	59.2	13.0	7	5.29	9.1
Harvest Queen	15837	6199	1	1	0	1	77	18	54	58	50	23	25	32	25	80	10							3	5		27.2	69.6	39.1	17	5.27	8.5
Mediterranean selection	5933–23	11525	1	10	3	4																										
Do	3015–63		4	9	3	5																		70			17.6	110.0	16.0	5	5.17	8.9
Forward		6691											0	3	0		5	23									5.3	33.6	14.5	4	5.03	8.3
Berkeley Rock		8272													25		5							0			5.2	19.5	26.7	6	5.04	9.1
Currell		3326						23	25	15			8	15			10	24						14			15.0	46.2	32.5	2	5.02	9.3
Hybrid 128		4512								0		0						10									16.8	40.1	41.9	8	5.00	9.5
Valley		5923													15	90	5										3.3	5.5	60.0	3	4.98	9.1
Mediterranean selection	3015–81	10086	7	33	4	5						8															36.7	71.0	51.7	3	4.97	9.6
																											11.4	55.3	20.6	5	4.93	8.6

TABLE 6.—*Comparison of field lodging of wheat varieties grown at selected experiment stations with breaking strength and weight per unit length of culm at Denton, Tex.*—Continued

Variety	T. S. No.	C.I. No.	Percentage of lodging during indicated year at—																								Average	Average expressed as percentage of lodging in Blackhull (same experiments)	Average percentage of lodging in Blackhull (same experiments)	Crop years compared	Denton, Tex., 1934	
			Denton, Tex.				Manhattan, Kans.								Urbana, Ill.		New Brunswick, N. J.		Bozeman, Mont.				Lincoln, Nebr.		Woodward, Okla.	Fort Collins, Colo.							Breaking strength of culm	Weight per unit of culm
																	Nursery	Field plot															
			1931	1932	1933	1934	1927	1928	1929	1930	1931	1932	1934	1935	1924	1931	1928	1928	1931	1933	1934	1935	1932	1935	1935	1934	1935						
			Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*No.*	*Lbs.*	*G*
Sutton	15832	10053	4	13	3	5																					6.3	43.4	14.5	4	4.93	8.5
Kanred × Fulcaster selection	20401		2	3	0	3																					2.0	13.8	14.5	4	4.86	8.4
Shepherd		6163								0	0						20										6.7	9.9	68.0	3	4.84	9.3
Minturki	20483	6155					72	0	0	27		6	0	3		60	20		23	2	30	77		40				25.7	51.7	49.7	14	4.82	8.4
Michikof		6990						0	52	50					10	80	20	46									36.9	64.4	57.3	7	4.81	8.3
Mediterranean selection	5933–20	10085	6	13	13	18						14	13													82	22.7	83.8	27.1	7	4.81	7.3
Mammoth Red		2008									15						25										20.0	34.8	57.5	2	4.79	9.1
Fulhio		6999											0	3	0									0			.8	4.4	18.5	4	4.74	8.9
Gladden		5644											0	0	0	0	5							0			.8	2.4	34.0	6	4.74	8.4
White Mediterranean	15834	10023		10	3	44																					19.0	109.8	17.3	3	4.74	8.1
Kanred × Hard Federation	20416	11373		4	18	3		5	72	81	68	12	0	0						0	0	18		0	4	28	29	20.1	46.9	42.9	17	4.71	8.3
Sibley No. 81	20427	10084		18	1	26						23	23	28											34			21.9	117.8	18.6	7	4.71	8.5
Red Rock		5597									10		0	10				30	13					5				11.3	32.3	35.0	6	4.65	8.5
Mediterranean selection	5933–34	11526	5	9	5	16																					8.8	60.7	14.5	4	4.60	7.1
Poole		3488						0					0	20	0		10	19						0			7.0	26.2	26.7	7	4.56	8.2
Kanred selection	20415	10099		28	T [1]	33						23	7	32										64	39	96	52	37.4	125.9	29.7	10	4.54	7.5
Cooperatorka		8861						0	91	72	62	17	0	20											19			35.1	79.6	44.1	8	4.49	8.1
Minhardi		5149							0	0		0	0	2		10	5		12	0	0	93		5				10.6	21.5	49.4	12	4.48	9.3

Kawvale	12577	8180	1	1	0	0	----	25	75	83	70	12	0	6	----	----	----	----	9	0	----	----	----	40	21	----	----	22.9	69.6	32.9	15	4.41	8.4
Early Blackhull	15838	8856	4	2	4	15	----	35	93	75	----	30	0	4	----	----	----	----	37	----	----	----	0	3	4	65	59	26.9	79.6	33.8	16	4.37	7.5
Fultz	20474	3416	6	4	----	61	----	0	----	70	20	----	0	----	----	----	10	42	----	----	----	----	----	----	----	----	----	23.7	64.4	36.8	9	4.33	8.1
Tenmarq	12578	6936	2	13	3	21	69	28	81	86	58	11	2	7	----	40	----	----	20	----	----	----	----	25	8	----	----	29.6	74.4	39.8	16	4.33	8.1
Trumbull	-------	5657	----	----	----	----	----	----	----	----	----	----	0	3	0	----	10	19	----	----	----	----	----	0	----	----	----	5.3	19.9	26.7	6	4.32	7.6
Fulcaster	7082	6471	2	8	3	6	72	35	82	68	67	----	0	23	15	----	40	----	28	0	17	93	----	5	23	----	----	30.9	80.9	38.2	19	4.32	6.9
Ridit	-------	6703	----	----	----	----	73	0	----	0	----	0	0	5	----	----	20	----	17	0	0	43	----	23	-------	----	----	15.1	34.7	43.5	12	4.27	7.0
Mediterranean selection	3015–105–1	11587	1	21	1	18	----	----	----	----	----	----	0	18	----	----	----	----	----	----	----	----	----	47	15	57	26	20.4	79.1	25.8	10	4.26	7.9
Fulhard	20420	8257	----	5	2	4	----	34	88	84	43	6	0	29	----	----	----	----	----	----	----	----	0	2	16	50	46	27.3	71.7	38.1	15	4.225	7.8
Webster×Malakof selection	20410	----	----	----	14	78	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	-------	----	----	46.0	287.5	16.0	2	4.20	7.8
Beloglina selection	20424	8884	----	35	6	31	----	----	----	50	80	28	10	----	----	----	----	----	37	2	----	----	0	----	28	86	----	32.8	94.8	34.6	12	4.11	6.4
Mediterranean selection	3015–72	----	5	39	9	10	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	15.8	109.0	14.5	4	3.97	7.3
Iobred	20430	6934	----	----	0	----	----	70	0	40	----	----	0	20	----	100	10	----	----	----	----	----	----	----	----	----	----	30.0	66.1	45.4	8	3.94	7.1
Cheyenne	18566	8885	----	6	0	8	----	----	----	78	48	10	0	3	----	----	----	----	4	0	17	42	----	12	20	----	----	17.7	48.4	36.6	14	3.86	7.1
Blackhull	7172	6251	6	20	6	26	76	27	84	89	75	45	2	25	25	90	40	46	38	23	40	95	0	22	6	85	60	42.0	100.0	----	25	3.88	6.9
Kanred	11763	5146	5	35	4	73	76	53	89	93	74	45	23	45	75	100	50	----	----	----	33	98	----	56	18	----	----	55.0	130.6	42.1	19	3.82	6.3
Oro	20389	8220	----	6	0	----	----	22	79	72	83	6	----	9	----	----	----	----	6	1	0	75	----	65	13	----	----	21.2	73.4	42.5	14	3.76	6.4
Nebraska No. 60	15835	6250	1	19	0	16	70	0	60	----	48	22	7	33	----	----	----	----	35	0	33	95	37	17	18	56	15	29.1	76.4	38.1	20	3.69	6.2
Wisconsin Pedigree No. 2	-------	6683	----	----	----	----	----	----	----	----	----	----	0	20	----	70	----	50	----	----	----	----	----	44	-------	----	----	36.8	99.5	37.0	5	3.51	6.5
Turkey	-------	1558	----	----	----	----	69	28	81	86	58	11	22	53	15	100	15	----	----	----	----	----	----	----	29	----	----	47.3	97.1	48.7	12	3.48	6.1
Nebraska No. 28	15836	5147	4	0	0	0	61	31	63	----	----	----	0	0	----	----	5	----	----	----	----	----	----	0	-------	----	----	14.9	49.0	30.4	11	3.48	6.8
Early Kanred	-------	8261	----	----	----	----	88	78	85	71	70	----	----	----	----	----	----	----	----	----	----	----	----	----	0	----	----	65.3	109.7	59.5	6	3.30	6.6
Kharkof	16830	1442	4	10	6	26	72	22	78	77	82	23	3	45	----	----	----	----	21	0	7	87	----	13	31	78	53	36.9	86.8	42.5	20	3.21	5.1

[1] T=trace.

Table 7 presents data on lodging from the uniform variety yield tests of winter wheat in the Great Plains, sponsored by the United States Department of Agriculture. In this case the averages for lodging were calculated in percentages of Kharkof, a variety common to all tests. The correlation coefficient for lodging and breaking strength was -0.575, which is well above the 0.05 point (0.468) but not equal to the 0.01 point (0.590).

TABLE 7.—*Field lodging of hard red winter wheat varieties in uniform yield nursery tests at eight experiment stations, 1931–35* [1]

Variety	T. S. No.	C. I. No.	Percentage of lodging during indicated year at—																						Average	Average expressed as percentage of Kharkof (same experiments)	Average percentage of Kharkof in lodging (same experiments)	Crop years compared	Denton, Tex., 1933–34	
			Denton, Tex.			Goodwell, Okla.		Manhattan, Kans.							Akron, Colo.		Bozeman, Mont.				Lincoln, Nebr.	North Platte, Nebr.	Waseca, Minn.							Breaking strength of culm	Weight per unit of culm
			1931	1932	1934	1931	1932	1927	9128	1929	1930	1931	1932	1935	1934	1935	1931	1933	1934	1935	1935	1935	1932	1933	1935						
			Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*			*No.*	*Lb.*	*G*
Kharkof	16830	1442	5	25	29	1	2	1	4	34	10	23	42	8	0	1	17	3	97	73	76	0	100	0	32	25.3	100.0		23	3.62	7.0
Kanred	11763	5146	11	25	26	2	2	13	17	36	7	36	81	20	0	1	22	2			80	15	100	17	43	26.5	134.5	19.7	21	4.05	7.0
Nebraska No. 60	15835	6250	5	20	9	T	2					8	58	17	0	1	17	2	93	75	87	0	100	0	41	28.2	100.4	28.1	19	3.91	6.3
Denmarq	12578	6936	4	10	6	2	2	9	1	15	0	3	38	8	1	0					33	40				10.8	66.3	16.3	16	4.30	8.0
Blackhull	7172	6251	3	19	13	2	2	15	7	26	11	8	87	7	1	0					54	38				18.3	112.3	16.3	16	4.23	7.6
Early Blackhull	15838	8856	15		8	16	3		7	21	7	23	48	5	27	0					28					16.0	88.4	18.1	13	4.54	8.5
Quivira	15833	8886	8	11	5	2	4		1	18	0	10	2	13	1	0		4			22					6.7	38.3	17.5	15	5.08	9.3
Cheyenne	18566	8885									0	12	17	0	0	0			67	33	36	0				16.5	50.0	33.0	10	3.98	7.3
Pro		8220						0	3	6	7	38	55	0	0	0			90	67	66	3				25.8	90.9	28.4	13	3.98	6.7
Turkey		1558						1	4	43	10	23	63	13												22.4	128.7	17.4	7	3.48	6.1
Kanred×Marquis		10090																												
Cooperatorka		8861																												
Minturki		6155										18	12	5	0	0	6	0	63	70	66	1	50	0	50	24.4	72.4	33.7	14	4.65	8.4
Turkey selection	20374	10016												0	0	0			100	68	48	13			49	34.8	96.9	35.9	8	3.45	7.2
Clarkan	20400	8858			3							32	50	12							17					22.8	64.0	35.6	5	6.71	12.0
Cawvale	12597	8180	0	T	1	2	2	10	0	10	10	0	17	0							48					7.7	38.5	20.0	13	4.92	9.3
Harvest Queen	15837	6199	4	3	6	4	3	1	0	9	3	10	53	17												9.4	61.4	15.3	12	6.01	10.2
Fulcaster	7082	6471	4	24	9	2	2	8	0	19	7	17	35	17												12.0	78.4	15.3	12	4.99	8.7
Nebraska No. 28	15836	5147	3	0		6	5																			3.5	42.2	8.3	4	4.60	8.6
Benton	9236	8265	5	2	3	2	2																			2.8	22.5	12.4	5	5.27	9.6

[1] QUISENBERRY, K. S. COMPARISON OF WINTER WHEAT VARIETIES GROWN IN COOPERATIVE PLOT AND NURSERY EXPERIMENTS IN THE HARD RED WINTER WHEAT REGION IN 1931, 1932, 1933, 1934, and 1935. U. S. Bureau Plant Industry, Division Cereal Crops and Diseases. [Unnumb. Pubs.] [Mimeographed.]

Further data are presented in table 8 for a different group of varieties grown at Pullman, Wash., Logan and Newton, Utah, Corvallis, Oreg., and New Brunswick, N. J. The averages for lodging were calculated in the same way as in the foregoing tests; Hybrid 128 was used as a standard of comparison.

TABLE 8.—*Comparison of lodging of wheat varieties for selected years and stations in the United States with strength of straw at Denton, Tex., 1934*

Variety	C. I. No.	Percentage of lodging during indicated year at—																		Average	Average expressed as percentage of lodging in Hybrid 128 (same experiments)	Average percentage of lodging in Hybrid 128 (same experiments)	Crop years compared	Denton, Tex., 1934	
		Pullman, Wash.									Logan, Utah				Newton, Utah				New Brunswick, N. J.	Corvallis, Oreg.				Breaking strength of culm	Weight per unit of culms
		Field plot test				Nursery test																			
		1931	1932	1933	1934	1930	1931	1932	1933	1934	1931	1932	1933	1934	1931	1932	1933	1934	1928	1933					
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*No.*	*Lb.*	*G*
Poppei	4238	0	0	0	-----	0	1	7	0	-----	----	----	----	----	----	----	----	----	5	-------	1.6	88.9	1.8	8	6.49	10.9
Goldcoin	4156	----	----	----	----	----	----	----	----	-----	16	12	20	8	----	----	----	----	5	0	10.2	100.0	10.2	6	5.97	9.9
Jones Fife	4466	0	14	2	49	3	3	4	2	100	----	----	----	----	----	----	----	----	-------	-------	19.7	88.3	22.3	9	5.80	10.8
Triplet	5408	0	2	2	55	3	33	4	2	100	15	23	17	13	15	23	17	13	20	20	18.3	110.9	16.5	19	5.52	11.8
Silvercoin	6013	----	----	----	-----	----	----	----	----	-----	16	10	17	8	----	----	----	----	10	-------	12.2	100.0	12.2	5	5.49	9.3
White Winter	5219	----	----	----	-----	----	0	0	1	-----	----	----	----	----	15	15	17	----	-------	0	6.9	113.1	6.1	7	5.11	9.2
Odessa	4475	----	----	----	-----	----	----	----	----	-----	21	25	22	----	----	----	----	----	30	-------	24.5	184.2	13.3	4	5.02	10.5
Golden	1003	----	2	0	100	0	6	8	0	100	17	20	8	----	----	17	20	8	-------	0	20.4	107.9	18.9	15	5.02	10.1
Hybrid 128	4512	1	2	0	97	0	0	0	1	100	17	7	19	8	10	13	19	9	10	0	16.5	100.0	----------	19	4.98	9.1
Mosida	6688	----	----	----	-----	1	0	13	2	98	----	----	----	----	----	----	----	----	20	-------	22.3	120.5	18.5	6	4.94	8.9
Kidit	6703	4	3	2	100	0	1	17	2	100	16	18	20	15	17	18	20	15	20	25	21.7	131.5	16.5	19	4.27	7.0
White Odessa	4655	----	----	----	-----	3	0	9	2	100	22	16	17	----	----	----	----	----	5	-------	19.3	112.9	17.1	9	3.99	7.3
Oro	8220	----	----	----	-----	4	19	12	0	100	24	13	30	15	25	----	30	15	-------	50	25.9	177.4	14.6	13	3.76	6.4
Nebraska No. 60	6250	1	3	0	-----	----	----	6	0	-----	----	----	----	----	----	----	----	----	-------	-------	2.0	250.0	.8	5	3.69	6.2
Sherman	4430	----	----	----	-----	----	----	----	----	-----	30	23	30	----	28	23	30	----	20	30	26.8	225.2	11.9	8	3.57	6.0
Kharkof	1442	1	9	1	100	6	0	11	1	100	25	23	30	20	25	23	30	20	-------	75	27.8	165.5	16.8	18	3.21	5.1

The correlation coefficient for average lodging at these stations and the breaking strength of straw at Denton was −0.729, which is highly significant.

These data clearly indicate that the very low coefficients for lodging and breaking strength given in table 5 are in part due to the paucity of data, especially with respect to lodging, and that when lodging data for a number of years or a number of stations are combined relatively high coefficients may be expected. This may explain the failure of some investigators to secure a significant correlation between lodging and the breaking strength of straw.

The fact that lodging is dependent on a number of factors, some at least varying from year to year, and that the correlation coefficients between lodging and breaking strength of straw increase with an increase in the number of years or number of stations included in the determinations suggests that the true relation between lodging and breaking strength of straw, as indicated by the correlation coefficients so far available, is underestimated. In other words, more extensive data than those now available, especially with reference to lodging, would probably provide even higher coefficients and consequently lead to placing greater confidence in breaking strength of straw as an adequate measure of tendency to lodge. Altogether, it would seem that there are sufficient data to support the belief that breaking-strength determinations can be used to predict lodging behavior in the field and consequently can be used in a breeding program to isolate strains resistant to lodging.

INTERANNUAL AND INTERSTATION CORRELATIONS, 1931–34

Interannual correlation coefficients for lodging and for breaking strength have been calculated for such varieties as were grown 2 or more years. The correlation coefficients are given in table 9. Only one of the coefficients for lodging, viz, for the period from 1932–34, is significant, whereas all those for breaking strength of straw are significant. In other words, so far as these data show there appears to be only a slight tendency for varieties to lodge the same from year to year as contrasted with a very strong tendency for them to have the same relative breaking strength of straw.

TABLE 9.—*Interannual correlation coefficients for lodging and breaking strength of straw*

Character correlated and year	Varieties	1932		1933		1934	
		Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Lodging:	*Number*						
1933	30	+0.013	0.95				
1934	30	+.566	<.01	+0.050	0.79		
Breaking strength of straw:							
1931	18	+.854	<.01	+.733	<.01	+0.632	<0.01
1932	65			+.756	<.01	+.816	<.01
1933	44					+.533	<.01

Interstation coefficients of breaking strength have also been calculated in some instances. Thus, for 15 varieties grown both at Denton, Tex., and Manhattan, Kans., during the 3-year period 1932–34, the

correlation coefficients for each individual year were 0.722, 0.346, and 0.58, respectively, and for the averages for all years, 0.787.

BREAKING STRENGTH OF STRAW AND WEIGHT PER UNIT LENGTH OF CULM

Breaking strength of straw, as may be noted in table 5, was correlated with height of plant and weight of grain per 100 heads and highly correlated with diameter of culm at the base of the plant, with weight of 100 culms, and with weight per unit length of culm at the base of the plant in each of the years in which these relations were studied. The chief interest lies in the possibility of substituting one of these determinations for breaking strength of straw as a measure of the tendency to lodge. The coefficients for breaking strength and weight per unit length of culm were especially high, viz, 0.956 and 0.911 for 1933 and 1934, respectively. The relation for 1934 is illustrated in figure 1.

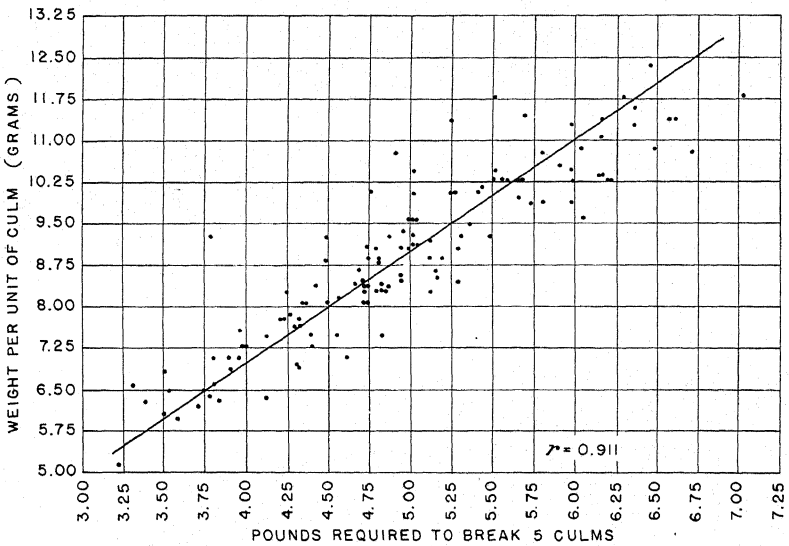


FIGURE 1.—Breaking strength of straw and weight per unit of culm at base of plant of 129 varieties of hard and soft winter wheats, Denton, Tex., 1934.

These coefficients are sufficiently high to suggest that weight per unit length of culm might safely be substituted for breaking-strength determinations. Such determinations would often be more convenient, as they do not involve the use of any special equipment, require less time, and in some respects may be expected to be more accurate. For one thing, the personal equation is involved to some extent in making breaking strength of straw determinations, as, for example, with respect to the speed with which the weight is lowered onto the straws. Errors of this sort are avoided in determinations of unit weight of culms.

As shown in table 5, there are no material differences in the coefficients between lodging and breaking strength of straw on the one hand and lodging and weight per unit length of culm on the other. In

no case are they statistically significant. For the data in table 6, the coefficient for lodging and weight per unit length of culm is −0.464, as compared with −0.422 for lodging and breaking strength. For the data in table 7, the corresponding coefficients are −0.625 and −0.575. For the data in table 8, the values are −0.666 and −0.729, respectively. For 36 varieties grown at Denton, in each of 2 years, the corresponding coefficients are −0.411 and −0.387.

The interannual coefficient for weight per unit length of culm in 1933 and 1934 is 0.586. This compares favorably with the corresponding coefficient (r=0.533) for breaking strength of straw for the same years (table 9). No data on weight per unit length of culm are available for other years or from other stations.

These results indicate that the weight per unit length of culm at the base of the plant is as good a measure of tendency to lodge as is breaking strength of straw. As already indicated, there are certain practical advantages in using the former.

SUMMARY

Varieties of winter wheat, varying in number from 18 to 129, were grown at the Texas Substation No. 6, Denton, Tex., in each of 4 years, and the relation of lodging to various plant characters, including breaking strength of straw and weight per unit length of culm, was studied. Data with respect to lodging for a number of the same varieties grown at Manhattan, Kans., New Brunswick, N. J., Pullman, Wash., Corvallis. Oreg., Logan and Newton, Utah, Urbana, Ill., Bozeman, Mont., Lincoln and North Platte, Nebr., Fort Collins and Akron, Colo., Waseca, Minn., and Woodward and Goodwell, Okla., were supplied by the agricultural experiment stations of the respective States, and the results were correlated with the breaking strength of straw and certain other characteristics of the same varieties as were grown at Denton.

Lodging was dependent on a number of factors that vary greatly from year to year, thus making it difficult to find any one index of lodging that may be considered completely reliable. A positive and significant though small correlation coefficient was recorded for lodging and length of lower internode and for lodging and date of maturity in one season only of the three in which these relations were studied; likewise, a positive and significant though small correlation coefficient was recorded for lodging and weight of grain per 100 heads in one season only of the two in which this relation was studied; and a small negative significant coefficient between lodging and weight per 100 culms and lodging and weight per unit length of culm in one season only of the two in which these relations were studied. All other coefficients, including lodging and breaking strength of straw, were small and were not statistically significant. Holding various factors constant by means of partial correlation coefficients raised the value of the coefficient for lodging and breaking strength of straw in some cases, but in no case did it reach the level of statistical significance.

When the average lodging for a number of years or a number of stations was correlated with breaking strength of straw for the same varieties as were grown at Denton, the coefficients were in all cases significant, and in most cases highly so. These results suggest that

the low coefficients sometimes secured for lodging and breaking strength of straw are due to the numerous factors that determine lodging in any single case, and they also suggest that failures to demonstrate a significant relation between lodging and breaking strength of straw are due in the main to lack of sufficient data with respect to lodging. In any event, the relation between breaking strength of straw and tendency to lodge appears to be such as to justify the use of the former in evaluating new varieties with respect to the tendency to lodge, particularly in a breeding program the purpose of which is to secure varieties resistant to lodging.

Relative breaking strength of straw is shown to be fairly constant from year to year, whereas lodging is not, from which it would appear that determinations of breaking strength for a single season are a more reliable index of tendency to lodging than is a record of lodging itself for a single season.

Correlation coefficients between lodging and weight per unit length of culm taken near the base of the plants were as high as, or higher than, those between lodging and breaking strength of straw. Determinations of the former require no special equipment, are more quickly and easily made, and in some respects are believed to be more accurate. It is therefore suggested that weight per unit length of culm near the base may be used to advantage, instead of breaking strength of straw, as an index of lodging.

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EFFECT OF CARBON DIOXIDE STORAGE ON BARTLETT PEARS UNDER SIMULATED TRANSIT CONDITIONS[1]

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INTRODUCTION

The Bartlett is by far the most popular pear variety grown in the United States. Its commercial production is confined largely to the three Pacific Coast States, where approximately 80 percent of the total pear acreage is planted to this variety (*16*).[2]

The transportation of a large portion of this Bartlett pear tonnage to eastern markets should be accomplished with little loss of storage capacity and dessert quality. This variety is stored and transported best at temperatures of 29° to 30° F. (*17*), but during transportation and market distribution it is difficult to maintain temperatures below 40°, and temperatures much higher than this are likely to prevail. Even with precooled fruit it is not possible at present to keep the load cool enough in all parts of a refrigerator car to prevent considerable ripening. When subjected to temperatures between 40° and 50°, Bartlett pears soon show a yellow ground color, ripen rapidly, and break down at the core. Consequently they frequently undergo these changes before reaching the consumer.

The storage life of many fruits and vegetables may be considerably extended by increasing the concentration of carbon dioxide gas in the storage atmosphere (*1*, *14*). The data presented in this paper are based on a study of the storage and ripening behavior of Bartlett pears when refrigeration has been supplemented by the use of carbon dioxide gas. The tolerance of the fruit for this gas has been studied by varying the concentration, temperature, and period of exposure. Differences in degree of ripeness or firmness of the fruit under various storage practices have been measured in terms of soluble pectin. The experimental work here reported was done at Wenatchee, Wash., and represents one phase of a general investigation of the handling and storage of the Bartlett pear that is being carried on at this station (*6*, *7*, *9*, *10*).

REVIEW OF LITERATURE

No attempt will be made to present a thorough discussion of the rapidly increasing literature dealing with gas storage of fruits and vegetables. For such a discussion reference should be made to the reviews of Brooks et al. (*3*), Thornton (*25*), and Eaves (*5*).

Low-temperature disorders sometimes become limiting factors in the successful storage of apples at 32° F. (*14*). Kidd and West (*12*, *13*) have shown that in such instances satisfactory storage life can be preserved at higher temperatures by reducing the oxygen and increasing the carbon dioxide content of the storage air. The concentration

[1] Received for publication May 28, 1937; issued March, 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 134.

of these two gases in the storage air differed according to the variety of fruit under observation. For maximum storage life at 40°, Bramley Seedling required 11 to 13 percent of oxygen and 8 to 10 percent of carbon dioxide; Cox Orange Pippin, on the other hand responded best to atmospheres carrying 2.5 percent of oxygen and 5 of carbon dioxide.

Trout (*26*) studied the storage behavior of pears in atmospheres containing various concentrations of nitrogen and carbon dioxide at storage temperatures of 37.4°, 50.0°, and 64.4° F. At the two higher temperatures he obtained no beneficial results as compared with controls held in air. At 37.4° he was able to store pears successfully for 21 days in an oxygen-free atmosphere containing less than 10 percent of carbon dioxide. These pears ripened 6 weeks after being placed in storage as compared with 4 weeks for controls held in air; i. e., their storage life was prolonged by 50 percent. Kidd et al. (*15*) studied the storage behavior of Comice pears at 37.4° in artificial atmospheres ranging from 0 to 20 percent in carbon dioxide content and from 21 to 0.2 percent in oxygen content. These authors found that subnormal oxygen over a range of 21 to 2 percent produced only a slight retardation of ripening in atmospheres devoid of carbon dioxide and that "the higher the oxygen content the more sweet the ripened pear." A marked depression in the rate of ripening occurred when the oxygen level was lowered to 0.2 percent. Carbon dioxide in the presence of oxygen above 2 percent greatly retarded ripening; the degree of retardation varied with the concentration of carbon dioxide present. After storage in air at 37.4° for 84 days, Comice pears ripened in 8 days when removed to 50°; comparable fruit held in 10 percent of carbon dioxide and 6 percent of oxygen for a similar period ripened in 20 days, while that stored in 20 percent carbon dioxide required 27 days to ripen properly.

Working with Bartlett pears, Thornton (*25, p. 229*) obtained little benefit from the use of increased concentrations of carbon dioxide. He states:

> Unlike the Anjou variety, the freshly harvested Bartlett pear was greatly injured by storage for 7 days in 28 percent of carbon dioxide at all temperatures. The Bartlett pears in 10 percent of carbon dioxide were similar to the controls in color and firmness, but they were less astringent in flavor.

It is difficult to reconcile Thornton's results with those of other workers (*3, 15*) and with those obtained in the present investigation.

Brooks, Cooley, and Fisher (*2*) have shown that the carbon dioxide tolerance of Grimes Golden apples increased with a reduction in storage temperature. Flavor was unaltered by storage of the fruit in 100 percent of carbon dioxide for 6 days at 50° F., whereas injury resulted after 2 days at 86°. Magness and Diehl (*19*) obtained no appreciable effect upon the flavor of Winesap and Delicious apples by storing them for 10 days at 71.6° in 20 percent of oxygen and 5 to 10 percent of carbon dioxide; the rate of softening, however, was markedly retarded. Concentrations of 20-percent carbon dioxide or higher produced fermentative "off flavors." Thornton (*25*) has reported Delicious apples as being twice as tolerant as McIntosh to abnormal concentrations of carbon dioxide. No injury was observed in Delicious held for 7 days in 83-percent carbon dioxide at 32° or in 50 percent at 50°; McIntosh under similar conditions withstood carbon dioxide concentrations of 48 and 25 percent, respectively. Overholser

(*23*) found that 20 to 25 percent of carbon dioxide prevented softening of Fuerte avocados at 45° and no objectionable flavors were produced during or after storage. Fruit stored in concentrations of 4 to 5 percent of this gas at 45° could be held a month longer than the control.

MATERIAL AND METHODS

Bartlett pear fruits were selected for uniformity of size and maturity from typical, vigorous, 27-year-old trees growing under comparable orchard conditions. The fruit was harvested at optimum maturity, as indicated for each season, washed in cold 1-percent hydrochloric acid, wrapped in oiled paper, packed in standard 44-pound pear boxes, and placed immediately under experimental storage conditions. Fruit destined for carbon dioxide storage was placed in galvanized-metal chambers, 26 cubic feet in capacity, and each was fitted with a flanged edge and a water seal in which the cover rested. Copper tubes in the cover facilitated transfer and measurement of the gases. A small electric fan mounted inside the cabinets circulated the enclosed atmosphere, prevented stratification, and facilitated carbon-dioxide measurements. The carbon dioxide was obtained from cylinders of compressed gas. Atmospheric measurements of the gas were made with an Orsat-Fischer gas apparatus. The carbon-dioxide concentrations did not vary more than 2 percent during the course of any experiment. No attempt was made to depress the concentration of oxygen below that which would normally follow replacement during the addition of specified amounts of carbon dioxide. The maintenance of still lower oxygen concentrations appears to be impracticable under present commercial transit conditions; furthermore, as shown by Kidd et al. (*15*), the beneficial results therefrom would be of little importance.

Fruit destined for ripening studies was removed to 65° F. and a relative humidity of approximately 85 percent. Pressure tests of firmness were made on the pared flesh of 10 representative fruits by means of the $\frac{5}{16}$-inch plunger of a pressure tester developed by the United States Department of Agriculture. Soluble-pectin determinations were made on 100 g of juice after clarification and filtration through filter-cell. The method of precipitation and measurement of soluble pectin was essentially that of Carré and Haynes (*4*). Preliminary trials, in which 100 g of tissue were used as compared with 100 g of juice, substantiated the results reported by Haller (*8*), namely, that similar amounts of soluble pectin were obtained irrespective of the two sampling techniques used.

RESULTS FOR 1934

Preliminary work, involving a study of the storage quality and ripening behavior of Bartlett pears as influenced by various handling and storage practices, was begun in 1934. The pears were harvested on July 26 at 15.6 pounds' pressure. Certain lots of fruit were subjected to conditions similar to those the fruit would have encountered in transit (45° F. for 10 days), while others were given a short precooling or a short exposure to carbon dioxide prior to storage. Certain lots were held at a higher temperature (65°) in carbon dioxide before they were stored at 32°. Fruit from all these treatments was compared with fruit that had been stored immediately in air at

32°. Pressure test, condition, and dessert quality of the various lots were compared at intervals during storage at 32° and during subsequent ripening at 65°. Pertinent data are presented in table 1.

TABLE 1.—*Condition and dessert quality of Bartlett pears in 1934 as influenced by various handling practices at harvest and by periods of storage at 32° F.*

Handling practice	Storage at 32° F.	Pressure test	Color and condition	Ripening period at 65° F.	Surface scald or core break-down	Dessert quality
	Days	*Lb.*		*Days*		
Immediate storage at 32° F.	0	15.5	Dark harvest green, excellent.	11	None	Excellent.
	38	15.0	Dark green, excellent	5	do	Do.
	79	14.4	Light green, some yellow	5	do	Good.
45° F. for 10 days, then 32°.	0	9.7	Light green, very good	4	do	Excellent.
	28	4.4	Yellow, firm, hard ripe	4	do	Good.
	69	3.3	Yellow, surface scald, poor.	5	Scald, core break-down.	Poor.
Precooled at 32° F. for 24 hours, 45° for 10 days, then 32°.	0	14.1	Green, very good	4	None	Excellent.
	28	7.0	Light green, some yellow	4	do	Very good.
	69	6.8	Full yellow, some scald	5	Scald, core break-down.	Poor.
65° F. for 24 hours in 35 percent CO_2, 45° for 10 days, then 32°.	0	10.6	Light green, very good	4	None	Excellent.
	28	5.7	Yellow, firm, hard ripe	4	do	Good.
	69	4.5	Full yellow, scald, poor	5	Scald, core break-down.	Poor.
65° F. for 10 days in 20 percent CO_2, then 32°.	0	15.5	Dark green, excellent	6	None	Excellent.
	28	15.2	Light green, very good	4	do	Good.
	69	15.5	Full yellow, no scald	5	do	Fair to poor.
65° F. for 10 days (check)	0	------	Full yellow, eating ripe	0	do	Excellent.

The dessert quality of all lots, irrespective of handling practice was excellent when the fruit was ripened immediately after treatment. This was still true of the different lots after a storage period of 28 days at 32° F. Differences in potential storage life were great at this time, however, as shown in table 1. Transit temperatures of 45° for 10 days, even when supplemented by precooling or pregassing for 24 hours, greatly stimulated the ripening processes; precooling, however, was more beneficial than gas treatment. The potential storage life of these lots held at 45° for 10 days was decreased over 60 percent as compared to similar fruit stored immediately at 32°.

Pears held in air for 10 days at 65° F. following harvest were "eating" ripe. Those held in 20-percent carbon dioxide under similar conditions were dark green and appeared comparable in color and firmness to those stored immediately at 32°. Subsequent softening of the gas-treated fruit was retarded by air storage at 32°, as is shown by the pressure test after 28 days' storage at this temperature. Dessert quality and flavor of the ripened gas-stored fruit was good after 28 days at 32°; after 69 days, although no scald or core breakdown had occurred, the flavor was definitely impaired.

Fruit held at 45° F. for 10 days at harvest and stored for 69 days at 32° became overripe, badly scalded, and broken down at the core even in cold storage; senescence was decidedly more advanced than in comparable lots held for 10 days at 65° in 20 percent of carbon dioxide.

RESULTS FOR 1935

Storage investigations were undertaken during 1935 to determine the supplementary value of carbon dioxide in the handling of Bartlett pears under conditions similar to those the fruit would have encountered in transit. Two lots of pears were held for 10 days in 20 per cent of the gas at temperatures of 45° and 65° F., respectively. A control lot for the fruit held at each of the above-mentioned temperatures and one lot that was stored immediately at 32° were used for comparative observations. The fruit was harvested on August 20 at 17.3 pounds' pressure and placed under experimental storage conditions within 4 hours from the time of picking. After 10 days at the higher temperatures, it was placed at 32° and withdrawals were made at irregular intervals for studies on ripening, pressure test, and dessert quality. The data pertaining to these observations are presented in table 2.

TABLE 2.—*Storage and ripening capacity of Bartlett pears in 1935 as influenced by various handling practices at harvest and by periods of storage at 32° F.*

Handling practice	Storage at 32° F.	Pressure test	Color and condition	Ripening period at 65° F.	Surface scald or core break-down	Dessert quality	Additional period of edibility
	Days	*Lbs.*		*Days*			*Days*
Immediate storage at 32° F.	10	18.1	Dark harvest green, excellent.	8	None	Excellent	6
	50	17.1	Dark green, excellent	5	do	do	3
	72	16.3	Light green, slight yellow, very good.	5	do	Very good	2
	102	16.0	Very light green, much yellow, fair	6	Core break-down, no scald.	Poor	0
45° F. for 10 days, then 32°	0	17.0	Light green, very good	6	None	Very good	4
	40	14.2	Almost full yellow, fair	5	Core break-down, no scald.	Poor	0
	62	13.4	Full yellow, scald, and core break-down.	----	----	----	----
45° F. for 10 days in 20 percent CO_2, then 32°	0	17.1	Dark harvest green, excellent.	8	None	Excellent	6
	40	16.3	Green, no yellow, very good.	5	do	Very good	2
	62	16.5	Light green, some yellow, good.	5	do	do	2
	92	15.4	Similar to fruit given immediate storage at 32°.	6	Core break-down, no scald.	Poor	0
65° F. for 10 days in 20 percent CO_2, then 32°.	0	17.0	Dark green, excellent	8	None	Excellent	6
	40	16.3	Similar to lots delayed at 45° for 10 days.	5	do	Fair, slight alcoholic taste.	1
	62	16.6	Full yellow, no scald or core break-down.	5	do	Poor, off flavor	0
65° F. for 10 days (check).	0	----	Full yellow, eating ripe	0	do	Excellent	8

Here again "transit" conditions (45° F. for 10 days) greatly lowered the potential storage life of the fruit. Carbon dioxide storage at 65° for 10 days was decidedly more beneficial than air storage at 45°. Fruit held for 10 days in 20 percent of carbon dioxide at 65° was in fair condition, free from scald or core break-down and of fairly edible quality (slightly alcoholic taste) after 40 days' storage at 32°, while that delayed for 10 days at 45° in air was of poor dessert quality and showed core break-down but no surface scald after a similar period of storage at 32°.

The addition of 20 percent of carbon dioxide to the storage atmosphere during delay at 45° F. so retarded ripening and the development of yellow ground color that fruit treated in this manner compared favorably in dessert quality and potential storage life with similar fruit stored immediately at 32°. The efficiency of carbon dioxide storage varied with the temperature. Fruit stored in this gas at 45° was decidedly superior to similar lots stored in gas at 65°. These differences were reflected in the color and condition of the fruit in subsequent cold storage, as shown in table 2. Carbon dioxide storage at 65°, however, was preferable to air storage at 45°.

RESULTS FOR 1936

GAS STORAGE EXPERIMENTS

During 1936 more detailed consideration was given to certain problems associated with carbon dioxide storage of Bartlett pears. Fruit for these studies was harvested August 13 and gave a pressure test of 17.6 pounds; it was placed immediately under experimental storage conditions. It should be recognized that the greatest practical benefits of gas treatment depend upon its use at storage temperatures higher than 32° F. Since transit temperatures for pears approximate 45° more nearly than 32°, the higher temperature received major consideration in the following experiments. For reasons more technical than practical, gas-storage studies were also made at 65° in 20 percent gas for 10 to 30 days. The tolerance of Bartlett pears to carbon dioxide at 45° was determined by varying the concentration of gas from 10 to 35 percent and the time from 10 to 30 days. Check lots of fruit were air-stored immediately at 32° and after various periods of delay at 45°.

All the delayed lots of fruit were stored in air at 32° F. for further comparison of their potential storage life and dessert quality. Withdrawals were made at irregular intervals and the fruit was ripened at 65°. Data relative to the firmness, color, and condition of the various lots immediately after delayed storage and before subsequent storage at 32° are presented in table 3.

TABLE 3.—*Color and condition of Bartlett pears in 1936 as influenced by various handling practices at harvest and prior to storage at 32° F.*

Handling practice	Pressure test	Color and condition
	Pounds	
Immediate storage at 32° F	17.6	Dark harvest green, excellent.
45° F. for 10 days	17.0	Dark green, excellent.
45° F. for 15 days	7.1	Medium to light green, some softening.
45° F. for 20 days	3.7	Greenish yellow to full yellow, hard ripe.
45° F. for 30 days		Full yellow, poor condition, severe scald, no breakdown.
45° F. for 10 days in 10 percent CO_2	18.0	Dark harvest green, excellent.
45° F. for 15 days in 10 percent CO_2	17.5	Medium green, excellent.
45° F. for 20 days in 10 percent CO_2	15.6	Light green, slight yellow, good.
45° F. for 30 days in 10 percent CO_2	16.0	Do.
45° F. for 10 days in 20 percent CO_2	18.2	Dark harvest green, excellent.
45° F. for 15 days in 20 percent CO_2	17.5	Do.
45° F. for 20 days in 20 percent CO_2	16.5	Do.
45° F. for 30 days in 20 percent CO_2	16.8	Light green, good.
45° F. for 10 days in 35 percent CO_2	17.8	Dark harvest green, excellent.
45° F. for 15 days in 35 percent CO_2	17.8	Do.
45° F. for 20 days in 35 percent CO_2	17.6	Do.
45° F. for 30 days in 35 percent CO_2	17.5	Do.
65° F. for 10 days in 20 percent CO_2	18.0	Dark harvest green, good condition, slightly tough.
65° F. for 15 days in 20 percent CO_2	12.0	Light green to faint yellow, tough texture.
65° F. for 20 days in 20 percent CO_2	5.1	Full yellow, hard ripe.
65° F. for 30 days in 20 percent CO_2		Full yellow, soft, stale alcoholic, severe beak-down.
65° F. for 10 days (check)		Full yellow, prime dessert quality.

The data in this table indicate the degree of change that may be anticipated during the movement of fruit to market under different handling practices. The rate of ripening in air storage was very rapid at 45° F., but was greatly retarded by the addition of 10 percent of carbon dioxide to the storage air. At the end of 20 days the pressure test for the fruit stored in air was 3.7 pounds, and for that stored in 10 percent of carbon dioxide 15.6 pounds. During storage at 45° for periods of 15 days or longer a greater decrease in the rate of ripening was brought about by the addition of 10 percent of carbon dioxide to the storage air than by increasing the concentration of the gas from 10 percent to 20 percent or from 20 percent to 35 percent. The change in color of the fruit during storage at 45° was markedly retarded by increasing the concentration of gas to 20 percent; little additional retardation of change in color was found when the concentration was increased to 35 percent.

Fruit became eating ripe after 10 days in air storage at 65° F. Comparable lots of fruit held in 20 percent carbon dioxide for a similar period were firm, dark green, and, so far as could be determined, compared favorably with those stored immediately at 32°. Fruit held in gas for 20 days under similar conditions, however, was eating ripe and of good dessert quality. When the period of gas storage at 65° was extended beyond 20 days, dessert quality was greatly impaired. Accumulation of fermentative "off flavors" was probably the result of anaerobic respiration.

After having received the various preliminary treatments at harvest as shown in table 3, the pears were placed in storage at 32° F. Observations on the condition and potential storage life of these fruits at this temperature were made at irregular intervals thereafter. These data are summarized in table 4.

Fruit stored immediately at 32° F. could be held for 80 days; lots receiving delayed storage at 45° for 10 days without gas were firm ripe in 38 days, while those held for 15 days under similar conditions gave evidence of considerable ripening after 5 days at 32°. Carbon dioxide, even in the smaller amount of 10 percent, greatly increased the potential storage life of Bartlett pears. This conclusion is substantiated by the data under the heading "Color and condition" in table 4.

When fruit was delayed in 20-percent and 35-percent concentrations of gas at 45° F. transit temperature, it had a subsequent keeping quality equal to that of fruit stored immediately at 32°; in some instances (35-percent gas for 30 days) it showed less change in color after prolonged storage at 32° than did similar fruit held continuously at the latter temperature.

Bartlett pears softened very rapidly when held in air at 65° F.; they were eating ripe after a period of 10 days. A decided retardation in the rate of ripening occurred at this temperature when 20 percent of carbon dioxide was added to the storage atmosphere. Fruit after 10 days in such carbon dioxide storage was harvest green in color and could be held for an additional period of 10 days at 32° without impairment of dessert quality. Changes associated with ripening increased, however, even in gas storage, when the period of delay was longer than 10 days at 65°. Surface scald and core break-down resulted when gas storage at 65° was extended to 30 days.

Air storage at 45° F. was also detrimental to the potential storage life of pears. Under these conditions, changes in color and firmness

were greater than during gas storage at 65°. Surface scald and core break-down were apparent when the fruit was delayed at the transit temperature of 45° for 15 days or longer. Even after a 10-day period of delay, the potential storage life at 32° was reduced to less than 38 days. The susceptibility of pears to surface scald and core break-down was greater in air storage at 45° than in gas storage at 65°.

TABLE 4.—*Color and condition of Bartlett pears in 1936 as influenced by various handling practices at harvest and by periods of storage at 32° F.*

Handling practice	Storage at 32° F.	Pressure test	Color and condition
	Days	*Pounds*	
Immediate storage at 32° F.	20	17.5	Dark green, excellent.
	48	16.7	Dark green, no yellow color, good appearance.
	80	17.1	Light green, some yellow ground color, good.
45° F. for 10 days, then 32°	10	15.0	Light green, no yellow color, very good.
	38	8.7	Full yellow color, firm, hard ripe.
	70	7.2	Full yellow color, hard ripe, severe surface scald.
45° F. for 15 days, then 32°	5	5.2	Light green, considerable yellow ground color.
	33	----------	Full yellow, severe surface scald and core break-down.
45° F. for 20 days, then 32°	28	----------	Do.
45° F. for 30 days, then 32°	18	----------	Do.
45° F. for 10 days in 10 percent CO_2, then 32°.	10	15.4	Green, no yellow color, very good.
	38	15.6	Do.
	70	17.0	Light green, some yellow, good.
45° F. for 15 days in 10 percent CO_2, then 32°.	5	15.2	Light green, faint yellow, very good.
	33	15.5	Light green, faint yellow, good.
	65	16.0	Full yellow color, fair.
45° F. for 20 days in 10 percent CO_2, then 32°.	28	15.0	Full yellow color, fair, no scald.
	60	11.2	Full yellow color, some softening, no scald.
45° F. for 30 days in 10 percent CO_2, then 32°.	18	12.0	Do.
	50	8.7	Full yellow color, hard ripe, no scald.
45° F. for 10 days in 20 percent CO_2, then 32°.	10	16.0	Dark harvest green, excellent.
	38	16.8	Dark green, excellent.
	70	17.2	Light green, better than fruit given immediate storage at 32° F.
45° F. for 15 days in 20 percent CO_2, then 32°.	5	15.5	Dark harvest green, excellent.
	33	16.0	Dark green, like fruit given immediate storage at 32° F.
	65	18.0	Light green, like fruit given immediate storage at 32° F.
45° F. for 20 days in 20 percent CO_2, then 32°.	28	16.5	Green, no yellow color, very good.
	60	16.8	Light green, like fruit given immediate storage at 32° F.
45° F. for 30 days in 20 percent CO_2, then 32°.	18	16.0	Light green, some yellow color, good.
	50	17.0	Full yellow, fair condition, no scald.
45° F. for 10 days in 35 percent CO_2, then 32°.	10	16.0	Dark harvest green, excellent.
	38	16.7	Darker green than fruit given immediate storage at 32° F.
	70	17.7	Dark green, very good.
45° F. for 15 days in 35 percent CO_2, then 32°.	5	16.1	Dark harvest green, excellent.
	33	16.8	Darker green than fruit given immediate storage at 32° F.
	65	17.0	Dark green, very good.
45° F. for 20 days in 35 percent CO_2, then 32°.	28	16.0	Darker green than fruit given immediate storage at 32° F.
	60	16.7	Dark green, very good.
45° F. for 30 days in 35 percent CO_2, then 32°.	18	16.4	Dark green, like fruit given immediate storage at 32° F.
	50	17.0	Dark green, very good.
65° F. for 10 days in 20 percent CO_2, then 32°.	10	16.8	Light green, no yellow color, good.
	38	15.0	Considerable yellow color, fair.
	70	14.4	Almost full yellow, some softening, no scald.
65° F. for 15 days in 20 percent CO_2, then 32°.	5	11.3	Considerable yellow color, some softening.
	33	9.5	Full yellow, marked softening, no scald.
65° F. for 20 days in 20 percent CO_2, then 32°.	28	4.0	Full yellow color, hard ripe, no scald nor break-down.
65° F. for 30 days in 20 percent CO_2, then 32°.	18	----------	Full yellow, soft mealy, surface scald and break-down.
65° F. for 10 days (check)	0	----------	Full yellow color, prime dessert quality.

The color, condition, and firmness of the unripened fruit as influenced by various handling practices and periods of storage have been presented in some detail in the preceding discussion of the results for 1936. Ripening studies were also made on comparable fruit held at

65° F. after storage at 32°. Data pertaining to differences in ripening capacity and dessert quality, observed at intervals, are shown in table 5.

Bartlett pears stored immediately at 32° F. could be ripened with very good dessert quality after 80 days' storage. No surface scald or core break-down developed. The additional period of edibility of this fruit after acquiring prime dessert quality decreased, however, with prolonged storage, from 7 to 2 days. Fruit held at 45° without carbon dioxide for longer than 15 days was so advanced in maturity as to be unfit for further storage at 32°; even that delayed for 10 days under these conditions was worthless after 38 days at the lower temperature.

TABLE 5.—*Ripening of Bartlett pears in 1936 as influenced by various handling practices at harvest and by periods of storage at 32° F.*

Handling practice	Storage at 32° F.	Ripening period at 65° F.	Surface scald or core break-down when ripened	Dessert quality	Additional period of edibility
	Days	*Days*			*Days*
Immediate storage at 32° F.	20	7	None	Excellent	7
	48	5	do	do	4
	80	5	do	Very good	2
45° F. for 10 days, then 32°	10	4	do	do	3
	38	4	Core break-down	Poor	0
45° F. for 15 days, then 32°	5	4	None	Fair, slightly mealy	1
45° F. for 20 days, then 32°	0	3	do	Fair, mealy	2
45° F. for 10 days in 10 percent CO_2, then 32°	10	6	do	Excellent	8
	38	5	do	Very good	2
	70	4	Slight core break-down	Poor to fair	0
45° F. for 15 days in 10 percent CO_2, then 32°	5	5	None	Excellent	8
	33	5	Slight core break-down	Poor to fair	0
	65	5	Core break-down	Poor	0
45° F. for 20 days in 10 percent CO_2, then 32°	0	5	None	Excellent	9
	28	5	Slight core break-down	Poor	0
	60	5	Severe core break-down	do	0
45° F. for 30 days in 10 percent CO_2, then 32°	18	5	Slight core break-down	do	0
	50	5	Severe core break-down	do	0
45° F. for 10 days in 20 percent CO_2, then 32°	10	7	None	Excellent	7
	38	5	do	Very good	4
	70	5	do	Good	1
45° F. for 15 days in 20 percent CO_2, then 32°	5	7	do	Excellent	7
	33	5	do	Very good	2
	65	5	do	Good	1
45° F. for 20 days in 20 percent CO_2, then 32°	0	6	do	Excellent	8
	28	5	do	Very good	2
	60	5	do	Poor, tough	1
45° F. for 30 days in 20 percent CO_2, then 32°	18	5	do	Very good	2
	50	5	Core break-down	Poor	0
45° F. for 10 days in 35 percent CO_2, then 32°	10	7	None	Excellent	3
	38	5	do	Very good	3
	70	5	do	Good, tart	2
45° F. for 15 days in 35 percent CO_2, then 32°	5	7	do	Excellent	3
	33	5	do	Very good	1
	65	5	do	Good	1
45° F. for 20 days in 35 percent CO_2, then 32°	0	7	do	Excellent	3
	28	6	do	Very good	1
	60	5	do	Good	1
45° F. for 30 days in 35 percent CO_2, then 32°	18	6	do	Very good	1
	50	5	do	Good	1
65° F. for 10 days in 20 percent CO_2, then 32°	10	5	do	Excellent	3
	38	5	do	Fair, slightly alcoholic	0
	70	5	Core break-down	Poor	0
65° F. for 15 days in 20 percent CO_2, then 32°	5	5	None	Very good	1
	33	5	Core break-down	Poor	0
65° F. for 20 days in 20 percent CO_2, then 32°	0	5	None	Very good	1
	33	5	Core break-down	Poor	0
65° F. for 30 days in 20 percent CO_2 then 32°	0	0	Severe core break-down	Poor, stale, alcoholic	0
65° F. for 10 days (check)	0	10	None	Excellent	8

The addition of 10 percent of carbon dioxide to the atmosphere during delay at 45° F. prolonged the storage life at 32°. This fact is shown in table 5 by a comparison of fruit so treated with the air-stored

lot after 38 days' storage. Carbon dioxide at a concentration of 10 percent during periods of delay of 15 and 20 days extended the period of edibility considerably in lots which were ripened either immediately after storage or 5 days thereafter. During periods of delay at 45° for more than 10 days, carbon dioxide at this concentration afforded little or no protection against core break-down in Bartlett pears destined for prolonged storage.

The use of carbon dioxide in higher concentrations of 20 and 35 percent, during delay at 45° F., added materially to the period of time during which Bartlett pears could be successfully stored and ripened. In dessert quality, flavor, and freedom from surface scald and core break-down, such fruit compared favorably with lots stored immediately at 32°. No significant differences in storage or ripening capacity were detected between lots receiving 20 percent or 35 percent of the gas during delay. The only apparent difference was one of color; fruit in 35 percent gas was a shade greener during storage. Evidently 20 percent of carbon dioxide was sufficient to effect the maximum retardation of the ripening processes at 45°. Concentrations up to 35 percent or higher constitute a wide range that may be tolerated without injurious results.

Bartlett pears delayed at 65° F. in 20 percent carbon dioxide for more than 10 days were unsuited for storage at low temperatures. However, fruit could be held for 20 days in gas at 65° and then ripened immediately and still retain good dessert quality. Core break-down and a disagreeable, stale, sour, alcoholic taste usually accompanied prolonged gas storage at 65°. The fruit subjected to gas for 10 days at 65° could be held for an additional period of 10 days at 32° and ripened with no impairment of flavor; dessert quality, however, was impaired after storage for 38 days.

CHANGES IN PECTIN CONTENT OF BARTLETT PEARS

Decided changes in physical condition occur in Bartlett pears as they ripen to prime dessert quality; the most pronounced of these is the change in firmness of the flesh. This firmness, as recorded by the pressure test, may decrease from 15 to 18 pounds in unripened green fruit to 1 or 2 pounds in fruit that is fully ripened. Insoluble pectic material in cellular structure is often associated with maintenance of tissue rigidity. Hydrolysis of insoluble protopectin to soluble forms may be intimately associated with the softening of the fruit during normal ripening. In order to study this relationship Bartlett pears were harvested at optimum maturity and stored immediately at 32° F. for 38 days. During subsequent ripening at 65°, analyses for soluble pectin were made; the results are presented in table 6 and figure 1.

TABLE 6.—*Changes in soluble pectin content of Bartlett pears during ripening at 65° F. subsequent to storage at 32° F. for 38 days*

Ripened at 65° F. (days)	Soluble pectin	Color and condition of ripening fruit
	Percent	
0	0.025	Dark green, excellent storage condition.
2	.125	Light green, very firm.
4	.815	Full yellow, firm ripe.
8	.460	Overripe, no tissue discoloration.
11	.280	Overripe, slight core break-down.

That there is a significant correlation between degree of ripeness and production of soluble pectin is evident from table 6, which shows that the maximum formation of soluble pectin occurred before the fruit became overripe. The decrease of soluble pectin during senescence was probably caused by the more rapid hydrolysis of soluble pectin to pectic acid than of protopectin to soluble pectin. The speed at which changes in soluble pectin occur may be noted from figure 1. The soluble pectin increased almost sixfold in 2 days and reached a maximum in 4 days, when the fruit was firm ripe. Soluble pectin then decreased rapidly prior to the appearance of core break-down.

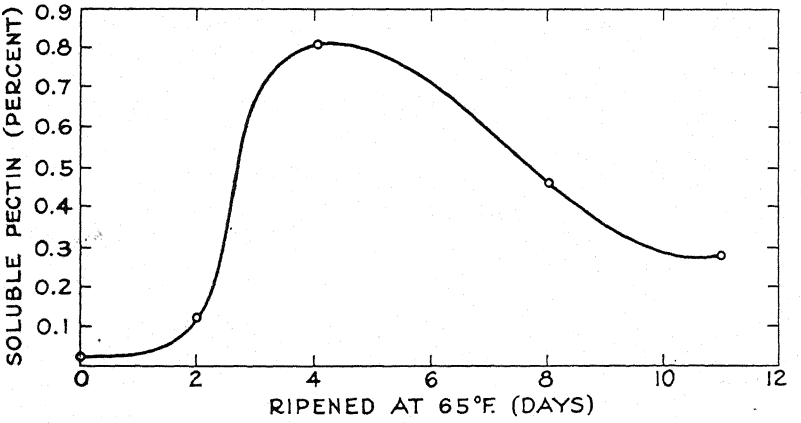


FIGURE 1.—Changes in soluble pectin content of Bartlett pears during ripening subsequent to storage at 32° F. for 38 days.

Since a significant correlation was found between degree of maturity and content of soluble pectin in Bartlett pears and since carbon dioxide has been shown to retard ripening, it became of physiological interest to study the effect of carbon dioxide storage on protopectin hydrolysis. Should gas storage depress the formation of soluble pectin, it would offer a possible explanation for the apparent efficiency of carbon dioxide in retarding the ripening of Bartlett pears. Data bearing on this subject are given in table 7.

The formation of soluble pectin was arrested in fruit that was stored immediately at 32° F. and held at this temperature for 13 or for 48 days. Ripening processes also were greatly retarded under similar circumstances.

TABLE 7.—*Soluble pectin content of Bartlett pears as influenced by various handling practices and by periods of storage at 32° F.*

Handling practice	Storage at 32° F.	Soluble pectin
	Days	*Percent*
Immediate storage at 32° F. (check)	13	0.0230
45° F. for 13 days in 20 percent CO_2	0	.0240
45° F. for 13 days	0	.3100
Immediate storage at 32° F (check)	48	.0250
45° F. for 13 days	33	.4720
45° F. for 13 days in 10 percent CO_2	33	.1000
45° F. for 13 days in 20 percent CO_2	33	.0325
45° F. for 13 days in 35 percent CO_2	33	.0287

Ripening progressed during delayed storage at 45° F. for 13 days and soluble pectin increased more than twelvefold to a concentration comparable with that in fruit ripened at 65° for several days (table 6). The soluble pectin in lots receiving such delayed storage increased about 52 percent when stored at 32° for 33 days. These facts substantiate practical storage observations that low temperature will not stop ripening in pears when once it has begun.

Determinations of soluble pectin were not made on the gas-stored lots immediately after treatment, but on similar lots after 33 days' storage at 32° F. From the data in table 7 it may be concluded that carbon dioxide definitely retarded ripening by depressing the hydrolytic (enzymatic) processes responsible for the formation of soluble pectin. The degree of retardation depended to some extent on the concentration of carbon dioxide. Gas concentrations of 20 and 35 percent gave soluble-pectin values comparable to those in fruit stored immediately at 32°. Data previously presented also indicate that these lots were similar in storage behavior and ripening capacity. It appears from the data presented in tables 6 and 7 that the degree of ripening and the potential storage life in Bartlett pears may be measured or predicted by evaluating the soluble-pectin content of the juice.

DISCUSSION

It should be recognized that the authors do not mean to recommend supplanting air storage of Bartlett pears at 30° to 31° F. with storage in artificial atmospheres of carbon dioxide at higher temperatures. The former practice should remain the accepted one whenever feasible. However, when sufficiently low temperatures are not available, as during transportation, congestion during harvest, or because of lack of refrigerated space, gas storage could be employed to great advantage as a supplement to low temperature in lengthening the storage life of Bartlett pears.

Magness and Ballard (*18*) and Magness et al. (*20*) have shown experimentally, and fruit auctions have evaluated commercially, a difference in the shipping and storage quality of Bartlett pears grown in various producing areas. Fruit from certain districts responds more readily to temperature stimulus than that from other districts, i. e., it will ripen more rapidly during a given length of time and in transportation to eastern markets will be in a more advanced state of ripeness on arrival. The potential storage life of this fruit could be greatly prolonged by the addition of carbon dioxide to the air in the car during transit.

From the viewpoint of commercial adaptability, it is fortunate that there is such a margin of safety in concentrations of CO_2 when Bartlett pears are exposed to an artificial atmosphere of this gas. Brooks et al. (*3*), using pony refrigerators, have determined the effective concentrations of this gas for storing Bartlett pears during periods of exposure up to 3 days at temperatures of 45° to 52° F. For Seckel pears these authors report no impairment of flavor or other evident injury after storage for 11 days at 66° in 50 percent of carbon dioxide, or for 17 days at 48° in a similar atmosphere. Thornton (*25*) has reported inability to successfully store Bartlett pears, obtained from a local wholesale dealer, for 7 days in 28 percent of carbon dioxide at temperatures of 32° to 50°. Because of the source of his material, however, it seems probable that the fruit might have been rather ripe

before it was placed in gas storage. If this were true it would explain the failure of Thornton's results to substantiate those of Brooks et al. (*3*), of Kidd, West, and Trout (*15*), and of the present authors, for when ripening once begins in pears it is exceedingly difficult to arrest it either by low temperature or by carbon dioxide storage.

The results herein reported establish for freshly harvested Bartlett pears a carbon dioxide tolerance that varies with storage temperature, concentration of carbon dioxide, length of exposure, and period of subsequent storage. Fruit destined for immediate consumption can be held at 45° F. in air for 15 to 20 days, in 10 percent gas for 20 to 30 days, and in 20- and 35-percent gas for 30 days or longer; and at 65° in air for 10 days and in 20-percent gas for 20 days. In each instance the fruit will ripen or may be ripened without impairment of flavor or dessert quality. Great differences, however, exist in the potential storage life of the fruit as a result of the various treatments. When it is desired to hold Bartlett pears for an additional period of storage at 32°, following intervals of exposure at harvest to various temperatures, periods of delay, or concentrations of gas, it becomes necessary to reevaluate their tolerance of these conditions. These investigations indicate that Bartlett pears can be held for the following periods in storage at 32°: 10 to 20 days after holding at 45° for 10 days; 38 to 40 days after holding at 45° in 10-percent of carbon dioxide for 10 days; 60 to 70 days after holding at 45° in 20-percent gas for 10 to 20 days; 60 to 70 days after holding at 45° in 35-percent gas up to 30 days; 10 to 20 days after holding at 65° in 20-percent gas for 10 days. It should be emphasized, however, that these gas tolerances represent maximum values and that they apply only to pears of best storage quality when harvested and handled under optimum conditions. In commercial practice, the industry would find it difficult to meet these standards; therefore, the period of gas storage and subsequent air storage at 32° would be proportionately less.

Bartlett pears have a tendency to develop severe surface scald when improperly handled (*10*). In the present investigations carbon dioxide always had a marked tendency to prevent the appearance of surface scald in the gas-stored fruit. This tendency persisted even during subsequent storage at 32° F. and during ripening at 65°. A recent report by Kidd, West, and Trout (*15*) substantiates these observations. Evidently carbon dioxide initiates some metabolic change in the fruit, following which normal ripening occurs without the appearance of surface scald. Acetaldehyde, as shown by Harley and Fisher (*10*), is usually associated with surface scald in Bartlett pears. There may be an interesting relation between carbon dioxide storage, depression of surface scald, and change in acetaldehyde content; such a suggestion, however, must also recognize the "CO_2-zymasis" work of Thomas (*24*), from the results of which he reports excessive accumulation of acetaldehyde in apples during storage in higher concentrations of this gas.

While Miller and Brooks (*21*) indicate that carbon dioxide treatment has no effect on the acidity of cherries, and Miller and Dowd (*22*) find no difference in the carbohydrate transformation of gas-stored peas, corn, and peaches, the results herein reported show that this gas does to a marked degree depress protopectin hydrolysis in pears. Such retardation from treatment in gas concentrations of 35 percent at 45° F. approximates that obtained by immediate storage at 32°.

Retardation of protopectin hydrolysis offers an explanation of the manner in which carbon dioxide may arrest softening and ripening of pears exposed to it. Although the suggestion was made by Hill (*11*) in 1913, this is the first instance, so far as the authors know, wherein carbon dioxide has been shown to retard markedly the formation of soluble pectin in stored fruit. A definite correlation also exists in pears between degree of ripeness and the amount of soluble pectin formed. From the data presented in tables 6 and 7, it is suggested that the amount of soluble pectin in Bartlett pears may be a significant index of the potential life of that fruit.

SUMMARY

This paper reports the results of storage, ripening, and soluble-pectin studies with Bartlett pears, in which temperature, concentration of carbon dioxide, and period of exposure have been varied.

When this variety was held at 45° F. for 20 days in 20 percent of carbon dioxide or for 30 days in 35 percent of carbon dioxide its potential storage life was not significantly different from that of fruit stored at 32° immediately after harvest. Freshly harvested fruit receiving such treatment may be subsequently stored at 32° and ripened at 65° without impairment of dessert quality.

The recommendation is made that whenever freshly harvested Bartlett pears are to be held at transit or storage temperatures materially higher than 30° to 32° F., and curtailment of ripening processes is desirable, carbon dioxide in a concentration of approximately 20 percent should be added to the storage air.

Carbon dioxide reduced surface scald in pear fruits.

There was a direct relation between the degree of ripeness and the amount of soluble pectin formed in Bartlett pears. Soluble pectin increased more than thirtyfold during ripening and then decreased with progressive senescence. Carbon dioxide retarded ripening by curtailing the processes associated with hydrolysis of protopectin. The soluble pectin content of fruit stored at 45° F. in 35 percent of carbon dioxide was comparable to that of fruit stored immediately at 32°.

The suggestion is made that a determination of soluble pectin in Bartlett pears may prove to be a valuable index of the degree of ripeness or of the potential storage life of the fruit at any given time.

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USE OF THE DILATOMETER IN MEASURING THE EXTENT OF FREEZING IN ICE CREAM AND RELATED PRODUCTS [1]

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INTRODUCTION

Although several workers have studied the extent to which ice creams are frozen at various temperatures, the results reported are incomplete, and the data are usually fragmentary. The present study, though likewise incomplete, is somewhat more extensive than previous reports in that samples of ice cream, ice milk,[3] and water ices (hereafter called simply "ices") have been included; in addition, observations have been made over a wider temperature range than in most of the other studies and the application of the dilatometer for measuring the extent of freezing seems to have certain advantages over the previous methods used. The results given in this report were obtained for the purpose of answering requests for more reliable information concerning the freezing of ice cream and related products.

HISTORICAL REVIEW

Various methods have been used in measuring or calculating the amount of ice formed at various temperatures in ice cream and related products. Hall (*9*),[4] observing temperature changes caused in a given amount of water by adding known amounts of ice cream at various temperatures, was able to approximate the amounts of ice formed in ice cream. Zoller (*15*) reported results obtained with the calorimeter, while Leighton (*11*), using the results reported by Pickering (*14*), devised a means of calculating the amount of ice formed on the basis of freezing-point determinations with mixes of known composition. Cole (*4*) separated the solid and liquid portions of ice-cream mixes at various temperatures either by centrifugation or by filtration under pressure, and arrived at values for the amount of ice formed over a limited temperature range.

Although the dilatometer has been widely utilized, in no report found by the author had it been used in measuring the amounts of ice formed in frozen dairy products. The fact, however, that Foote and Saxton (*6*, *7*, *8*) had determined with it the amount of water frozen in lampblack and inorganic hydrogels, and that Bouyoucos (*1*, *2*) had measured with it the amounts of soil moisture frozen at various temperatures, suggested its use in this study. This paper

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[2] The author is indebted to Bernice Fry, who made determinations on part of the experimental samples; to Dr. N. E. Edelfsen, irrigation division, University of California, who proposed helpful changes in the design of the dilatometer and in the manuscript during its preparation; to Dr. G. A. Richardson, dairy-industry division, and Dr. C. S. Bisson, chemistry division, University of California, for reading and constructively criticizing the manuscript; and to Dr. Max Kleiber, animal industry division, University of California, who did most of the glass blowing during the construction of the dilatometer, and whose criticisms of the theoretical considerations in the manuscript were very helpful.

[3] Ice milk is a frozen dairy product similar to ice cream except that its fat content is much lower. In California the minimum fat in ice milk is 4 percent; in ice cream, 10 percent.

[4] Reference is made by number (italic) to Literature Cited, p. 153.

reports some results obtained by measuring with a dilatometer the volume changes that accompany the freezing of ice cream and related products.

EXPERIMENTAL METHODS

The mixes used were prepared in accordance with commercial practices. Fresh milk, cream, and condensed skim milk were used as the sources of milk products; sucrose and gelatin as the other ingredients for ice cream and ice milk. No dairy products were used in the ices; but corn sugar was added along with the sucrose, and agar along with the gelatin. The ice mixes were heated sufficiently to dissolve the gelatin and agar used as stabilizers; the other samples were pasteurized at approximately 65° C. for 30 minutes, homogenized at pasteurization temperature, and cooled to about 5°. The ice-milk mixes were homogenized at 2,500 pounds pressure per square inch, a single-stage valve being employed. The double-stage homogenizer used for the ice-cream mixes was operated at 2,000 pounds pressure per square inch on the first stage and 750 pounds per square inch on the second stage for the mixes containing about 12 percent of fat; 1,800 pounds pressure per square inch on the first stage and 500 pounds per square inch on the second stage for the mixes containing about 18 percent of fat. The mixes were processed 1 or 2 days before being used in the dilatometer and were held at about 2° during this interim.

Since air in the sample would influence the volume changes accompanying freezing, the following precaution was taken before the sample was admitted to the dilatometer: It was placed under 25 to 28 inches of vacuum and heated sufficiently to start ebullition, then immediately cooled, the vacuum being maintained, and transferred to a weighing burette. This procedure did not completely eliminate, though it did reduce, the air in the samples. Since it was followed uniformly, however, the expansion factor used for the apparatus would partly correct for the errors introduced by this method.

The dilatometer used in these determinations is illustrated in figure 1. In making a determination compartment *a* was filled, to the mark indicated, with a portion of the sample. By opening the stopcock between compartments *a* and *b* and adjusting the leveling bulb, *d*, the desired amount of sample was admitted into compartment *b*. Then, after closing this stopcock, the weight of the sample in *b* was determined by refilling compartment *a* to the original mark from a weighing burette. Compartment *a* was then emptied by properly adjusting stopcock *e*. By means of the leveling bulb *d* and the three-way stopcock *f* the desired amount of mercury was admitted into capillary *c*, and stopcock *f* was then adjusted so that any change in volume in compartment *b* was measured by change in position of the mercury in the capillary.

The temperature of the sample was maintained at the desired levels by immersing the dilatometer in a brine bath so that the top of the sample in compartment *b* was 4 cm below the surface. The bath in turn was cooled by a refrigerated ice-cream cabinet in which it was placed. In certain instances solid carbon dioxide was added directly to the bath to hasten the cooling process. The temperature of the cabinet was maintained below that desired in the bath, but a thermo-

regulator was used to operate a heating element by means of which the bath temperature could be maintained at any desired point over the range 0° to −30° C. to within ±0.01°, or closer, as a result of supplementing the control by manual manipulation. Uniform temperature throughout the bath was assured by a motor-driven stirrer. For the temperature measurements over the range from −30° to approximately −10° a mercury thermometer graduated in 0.1° was used. It was found to be accurate to within one division on the scale. Over the range from approximately −10° to 0° or slightly higher,

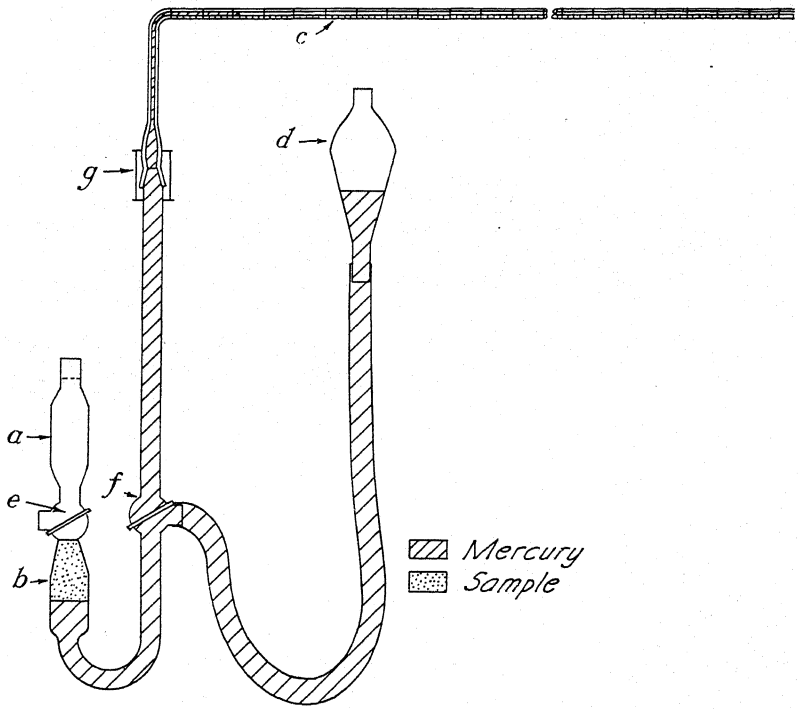


FIGURE 1.—The dilatometer: *a*, Compartment in which sample is measured; *b*, compartment in which sample is frozen; *c*, capillary tube in which the expansion is measured; *d*, leveling bulb; *e* and *f*, three-way stopcocks; *g*, ground-glass joint.

Heidenhain and Beckmann thermometers graduated in 0.01° were used.

Besides the sample of mix used in the dilatometer, other portions of the same mix were concentrated by evaporation under vacuum; and freezing-point and total-solids determinations were made on these as a basis of checking the results obtained in the dilatometer. With the ice mixes these concentrated portions were prepared by omitting part of the water required for the complete mixes. Although this second procedure was occasionally followed with ice-cream and ice-milk mixes, concentrated portions were also prepared by evaporating the samples according to the first method.

The freezing-point determinations in practically all cases were made with a Hortvet cryoscope. In a few instances, however, where the freezing points of the samples were low as a result of the mixes being

highly concentrated, a small portion of the sample was cooled in a brine bath until freezing began. Temperature readings taken at regular intervals were used in finding the freezing points of such samples. Duplicate or triplicate determinations were made in all cases.

In obtaining the dilatometer data, readings were taken at intervals of one-half hour or longer, and a sufficient number were taken at each temperature to make certain that no further changes in volume occurred. A constant reading was considered to indicate a state of equilibrium. Cooling curves did not deviate significantly from melting curves for the same samples.

Each sample which had been frozen and melted was supercooled and refrozen to determine whether or not the expansion the second

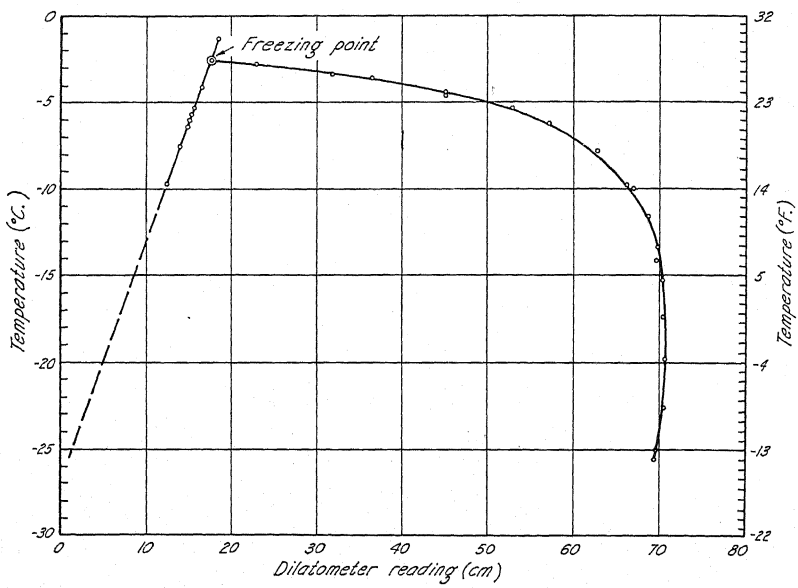


FIGURE 2.—Relation of dilatometer readings with supercooled sample to such readings after freezing. Sample M-7; 12.15 percent of fat, 38.14 percent of T. S.; freezing point, —2.55° C.; weight in dilatometer, 3.8504 g.

time would differ from the first. In a few cases samples were refrozen several times. For each sample reported, 30 to 50 points were carefully determined thus in the dilatometer as a basis for constructing the curve from which to calculate the amount of ice formed in the sample over the temperature range studied. Usually from 7 to 10 days were required for completing the measurements for each sample.

The basis for calculating the amount of ice formed in the sample under observation from the dilatometer readings is illustrated in figure 2, which represents graphically the dilatometer data obtained with sample M–7. The straight dashed line drawn through the points to the left represents data taken before the sample had been frozen and after it had been melted. Since most of these observations were made below the freezing point of the sample, the line is called the supercooled line. The points on the curve to the right represent readings obtained during both the freezing and the melting of the sample.

The horizontal distance at a given temperature from the supercooled line to the curve represents the linear expansion of the mercury capillary. This, multiplied by the factor representing the cross-sectional area of the capillary, gives the volumetric change that accompanies freezing at that temperature. Then, considering the size of the sample and the expansion factor representing the change in volume that accompanies the freezing of 1 g of water, the amount of ice formed at a given temperature is calculated. When a sufficient number of such values have been taken, another graph can be constructed as in figure 4 to represent the relation of temperature to the amount of ice formed. As will be shown presently, it was not difficult to get duplicate runs of the same mix to check in the dilatometer; but slight variations in the composition of mixes prepared at different times made these "duplicates" more difficult to check. All analyses for fat and total solids were made by the Mojonnier method and were run in duplicate.

EXPERIMENTAL RESULTS

STANDARDIZING THE APPARATUS

To standardize the dilatometer for subsequent use with experimental samples, the expansion factor accompanying the change of water into ice was determined. Water was boiled and cooled just before it was used for this purpose. The following expansion factors calculated as cubic centimeters per gram of water frozen were obtained: 0.0945, 0.0949, 0.0944, 0.0943, 0.0946.[5]

The close agreement of these values indicates the extent to which it was possible to duplicate results by this method.

As a further check on the dilatometer, a sucrose[6] solution (approximately 20 percent) was frozen in the apparatus. When the expansion factor of 0.0945, obtained with water, was used to calculate the amount of water frozen from this solution, the results differed from those calculated from International Critical Tables (*13, v. 4, p. 263*) data by about 1.3 percent. It was decided, therefore, to standardize the expansion factor for the dilatometer so that the results obtained with the sucrose solution would agree better with those computed from these data. An expansion factor of 0.0933 cubic centimeters per gram of water frozen gave close agreement (fig. 3). These results are tabulated as a convenience in calculating the amount of ice formed by taking into account freezing-point data on sucrose solutions (table 1).

TABLE 1.—*Relation of temperature to the amount of water frozen from a 20-percent sucrose solution and to the concentration of sucrose in the fraction remaining*

Temperature (° C.)	Water frozen	Ice in sample	Sucrose concentration in unfrozen fraction of sample		Temperature (° C.)	Water frozen	Ice in sample	Sucrose concentration in unfrozen fraction of sample	
	Percent	*Percent*	*Percent*	*Mols per 1,000 g water*		*Percent*	*Percent*	*Percent*	*Mols per 1,000 g water*
−1.49 [1]	0	0	20.0	0.736	−12.5	83.7	67.0	60.6	4.49
−2.0	23.0	18.4	24.5	.948	−15.0	86.2	69.0	64.5	5.30
−3.0	47.2	37.8	32.2	1.39	−17.5	87.4	69.9	66.4	5.77
−4.0	58.5	46.8	37.6	1.76	−20.0	88.9	71.1	69.2	6.56
−5.0	65.8	52.6	42.2	2.13	−22.5	89.5	71.6	70.5	6.97
−6.0	70.7	56.6	46.1	2.50	−25.0	90.2	72.1	71.7	7.40
−10.0	80.5	64.4	56.2	3.75	−26.8	90.6	72.5	72.7	7.77

[1] The freezing point of the solution was − 1.49° C.

[5] Three of these values were obtained by the author, and two by Bernice Fry, who assisted with part of this study.

[6] Baker's analyzed C. P. sucrose was used in preparing this solution.

Since the ice cream and related products included in this study resemble this solution more than they do water, and since the same temperature range was covered in both cases, the value of the expansion factor obtained with the sucrose solution was used as a basis for calculating the values given in this paper.

In presenting the data obtained with the experimental samples, the gross analyses for fat and total solids are given for each sample in

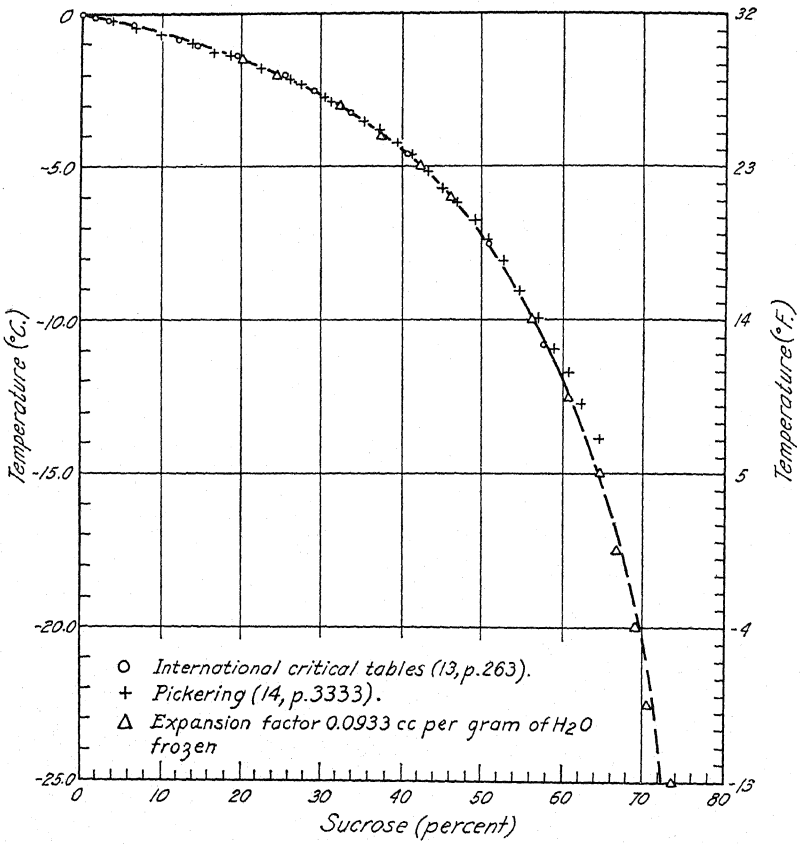


FIGURE 3.—Freezing-point lowering of sucrose solutions.

addition to its freezing point. The observations with the dilatometer and the freezing points on various concentrated portions of the same sample are in most instances presented graphically. These comparisons indicated a fairly satisfactory agreement between the two methods used. Especially is this true in the temperature range just below the original freezing points of the mixes. In some cases the agreement is good at temperatures as low as −15° C., but ordinarily the deviation here is greater than at the higher temperatures.

ICE-CREAM MIXES

Ice-cream mixes containing approximately 12 percent of fat and 38 percent of total solids are commonly used in making commercial ice

cream. In this study, measurements were made on five mixes of this type. Each was prepared separately, and although they were approximately the same in composition, each differed slightly from the others.

The data presented in table 2 were obtained with sample M–8 from duplicate runs in the dilatometer. These data are fairly representative of the group, and their close agreement indicates the precision of the method. The freezing-point determinations (not given in this table) also checked closely with the dilatometer data.

TABLE 2.—*Relation of temperature to the amount of water frozen in sample M–8* [1]

Temperature (° C.)	Water frozen		Ice in sample		Temperature (° C.)	Water frozen		Ice in sample	
	Trial 1	Trial 2	Trial 1	Trial 2		Trial 1	Trial 2	Trial 1	Trial 2
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
−2.47	0	0	0	0	−10.0	72.0	71.9	44.7	44.6
−3.0	18.4	18.1	11.4	11.2	−12.5	77.6	77.1	48.2	47.8
−3.5	28.3	28.2	17.5	17.5	−15.0	81.2	80.9	50.3	50.2
−4.0	36.0	35.8	22.3	22.2	−17.5	84.1	84.0	52.2	52.1
−5.0	48.0	47.6	29.8	29.5	−20.0	86.5	86.3	53.6	53.5
−6.0	55.6	55.2	34.5	34.3	−25.0	90.2	89.6	56.0	55.6
−7.5	63.5	63.3	39.4	39.2					

[1] The mix was found by analysis to contain 12.33 percent of fat and 37.98 percent of total solids. Its freezing point was −2.47° C. For the first and second determinations 4.902 and 4.648 g, respectively, were used.

The data from two other samples in this group are presented to show the agreement obtainable between observations on separate mixes which are very similar in composition. Table 3 gives the data secured with samples M–2 and M–7, the freezing points of which were −2.58° and −2.55° C., respectively. As can be seen also by comparing tables 2 and 3, some differences were found in the amount of ice formed in sample M–8 and in the two samples just mentioned. The differences apparent at the higher temperatures, however, become less significant in the lower temperature ranges.

Observations on other samples also indicated that samples having the same percentages of fat and total solids may have different freezing points, because of differences in the amount of soluble and ionizable constituents. In these cases the higher the freezing point of the mix the greater the amount of ice formed at a given temperature.

TABLE 3.—*Relation of temperature to the amount of water frozen in ice cream samples M–2 and M–7 containing approximately 12 percent of fat and 38 percent of total solids*

Temperature (° C.)	Water frozen in sample—		Ice in sample—		Temperature (° C.)	Water frozen in sample—		Ice in sample—	
	M–2 [1]	M–7	M–2	M–7		M–2 [1]	M–7	M–2	M–7
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
−3.0	13.1	13.1	8.1	8.1	−12.5	76.3	75.4	47.3	46.7
−3.5	24.5	23.1	15.2	14.3	−15.0	80.0	79.0	49.5	48.9
−4.0	33.2	31.4	20.6	19.4	−20.0	85.0	84.3	52.7	52.2
−5.0	45.2	45.2	28.0	28.0	−25.0	88.5	87.8	54.8	54.3
−7.5	61.4	61.0	38.0	37.7	−25.5	----	88.1	----	54.5
−10.0	70.8	69.8	43.8	43.2	−26.7	89.4	----	55.4	----

[1] Sample M–2 contained 12.0 percent of fat and 38.05 percent of total solids, and its freezing point was −2.58° C.; composition of M–7 given in legend of fig. 4.

The freezing-point data and the dilatometer measurements on sample M–7 (fig. 4) indicate the agreement possible between the two types of measurements on the same sample. Freezing-point determinations were not made on this sample below −7° C.

Under commercial conditions ice cream containing about 18 percent of fat and from 40 to 42 percent of total solids is manufactured and often sold as "catering" or "caterer's" ice cream. Observations were made on four mixes similar to this in composition. They varied

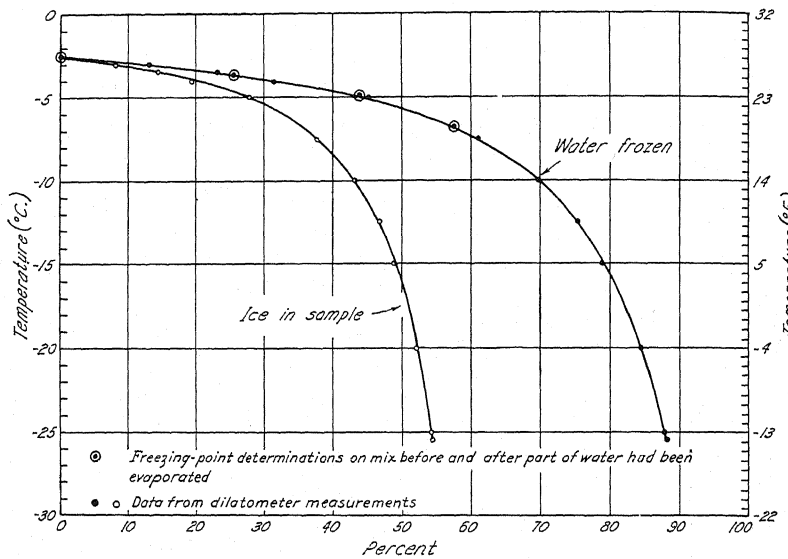


FIGURE 4.—Relation of temperature to percentage of water frozen and to percentage of ice in ice cream containing 12.15 percent of fat and 38.14 percent of total solids; freezing point, −2.55° C.; sample M–7.

enough so that the results from one sample could not be used as a check on the others. Figure 5 gives the results obtained with sample M–12. Freezing data agree closely with those secured with the dilatometer. The original freezing point of this mix, however, was higher than that of some others in this group. One should consider this fact before applying such data to other samples.

ICE-MILK MIXES

Ice milk, being comparable to low-fat ice creams, would be expected to give results resembling those obtained with such products. The results with two samples of ice milk having approximately the same composition are given here to confirm the statement. Table 4 shows the data for samples M–3 and M–6 to agree closely. Figure 6 gives the relation between the freezing-point data and those obtained with the dilatometer for sample M–3. Incidentally, the data approximate rather closely those given for ice-cream mix M–8 (table 2). Although these ice-cream and ice-milk samples differ widely in composition on the basis of analyses, their freezing points are −2.47° and −2.50° C., respectively. This fact probably explains the similarity in the results obtained.

TABLE 4.—*Relation of temperature to the amount of water frozen in ice milk in samples M–3 and M–6*

Temperature ° C.	Water frozen in sample—		Ice in sample—		Temperature ° C.	Water frozen in sample—		Ice in sample—	
	M–3	M–6 [1]	M–3	M–6 [1]		M–3	M–6 [1]	M–3	M–6 [1]
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
−3.0	17.4	17.9	11.7	12.0	−10.0	71.9	72.4	48.5	48.5
−3.5	27.6	28.3	18.6	19.0	−15.0	81.0	81.2	54.6	54.4
−4.0	35.8	36.2	24.2	24.3	−17.5	83.7	84.3	56.1	56.5
−5.0	47.6	47.9	32.1	32.1	−20.0	86.0	86.4	58.0	57.9
−6.0	55.3	55.6	37.1	37.3	−25.0	89.2	88.9	60.2	59.6
−7.5	63.0	63.5	42.5	42.6	−28.0	90.3	.....	60.9	.....

[1] M–6 contained 4.61 percent of fat and 32.99 percent of total solids. Its freezing point was −2.56° C. See legend of fig. 6 for composition of M–3.

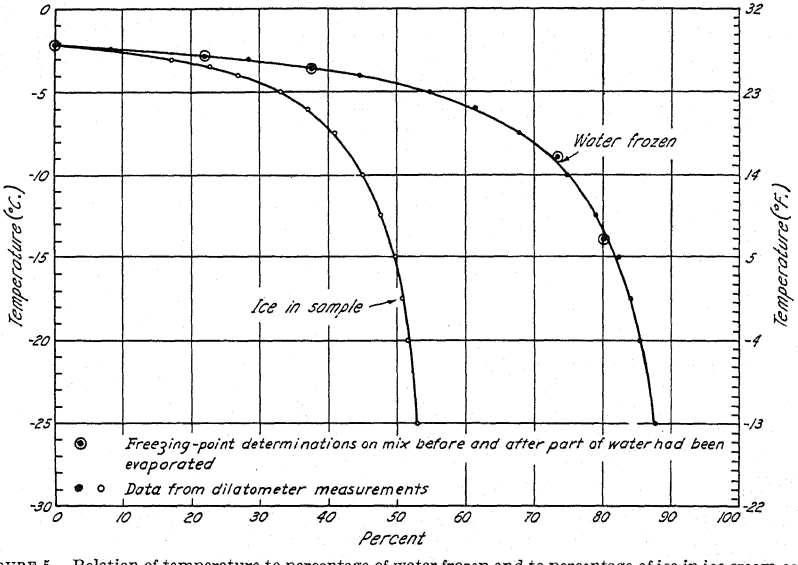


FIGURE 5.—Relation of temperature to percentage of water frozen and to percentage of ice in ice cream containing 18.68 percent of fat and 39.68 percent of total solids; freezing point, −2.13° C.; sample M–12.

ICE MIXES

Most manufacturers of ice cream make, in addition, ices or sherbets, or both. Sherbet mixes, however, are often made by simply adding ice-cream mix or other milk products to the mixes used in making ices. It seemed best, therefore, to obtain data for ices, since the results for sherbets would most likely be intermediate between those for ices and ice cream. The results obtained may not have general application, since considerable variation occurs in the composition of ices as made commercially. Some contain sucrose alone as the sweetening agent; others, combinations of sugars, such as sucrose and glucose. The composition may vary in other respects also, as, for example, in the addition of fruits, which are ordinarily used for flavoring.

Observations were made on four ice mixes prepared to contain approximately 23 percent of sucrose, 7 percent of glucose (corn sugar),

0.2 percent of gelatin, 0.2 percent of agar, and 0.5 percent of titratable acidity, calculated as citric acid. The results for one of these samples are given in figure 7. They differ markedly from those obtained with ice cream and ice milk, in that less water is frozen at a given temperature for the ices. The comparatively low freezing points of the ice mixes account for this difference.

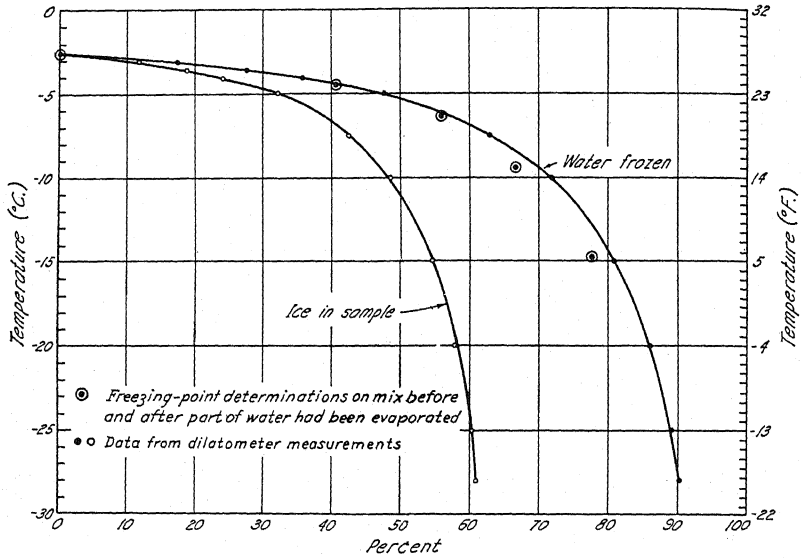


FIGURE 6.—Relation of temperature to percentage of water frozen and to percentage of ice in ice milk containing 4.55 percent fat and 32.55 percent total solids; freezing point, −2.50° C.; sample M-3.

THEORETICAL CONSIDERATIONS CONCERNING CHANGES IN VOLUME ACCOMPANYING FREEZING

The freezing process causes marked changes in concentration with respect to the substances in solution in the unfrozen portion of the sample. A given amount of solute is known to change the volume of a solution to which it is added to an extent which in some instances depends upon the concentration of the solution. Conceivably such changes might explain part of the changes in volume measured by the dilatometer during freezing.

These changes can be evaluated by a consideration of partial molal volumes or partial specific volumes, or they may be calculated directly from adequate specific volume data.

As a basis for understanding what changes occur during freezing let us start with 100 g of a 20-percent sucrose solution. Assume now that we remove successively from this solution either 5-g or 10-g quantities of water until the remaining solution consists of 20 g of sucrose and 5 g of water. (See columns 1 to 3, inclusive, of table 5.) If we assume further that these changes occur at constant temperature, then we can calculate the changes in volume which have occurred by taking into account the specific volumes and weights of the solution remaining at the various stages in the process.

Data for the specific volumes of sucrose solutions are not available over the temperature range desired, but they can be calculated for 20° C. from density data as reported in the International Critical

Tables *(13, v. 2, pp. 343–344)*. These values are given in column 4 of table 5. Utilizing such data, calculations were made to indicate the influence of concentration upon the change in volume of the solution. The results of these calculations are tabulated in columns 5 and 6.

In addition, either equation (1) or (2) given below can be used as a basis for calculating the values in columns 5 and 6. Using equation (1) it is necessary to evaluate the partial specific volumes or partial molal volumes of both components of the system, whereas with equation (2) only the partial molal volume of water need be evaluated. This was

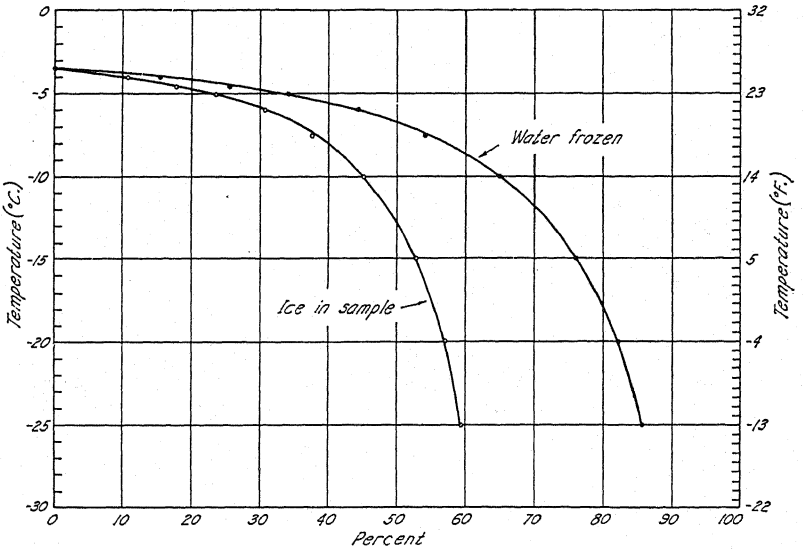


FIGURE 7.—Relation of temperature to percentage of water frozen and to percentage of ice in water ice. Sample M-16: No fat; 30.615 percent of total solids; freezing point, −3.48° C.

done by the method of intercepts as reported by Lewis and Randall *(12, pp. 38–40)*, using the specific volume data previously mentioned.

At any stage in the process the volume can be calculated by equation (1), assuming the temperature to be constant.

$$V=n_1\overline{V}_1+n_2\overline{V}_2 \qquad (1)$$

Where

V =volume of the solution

$\overline{V}_1=\frac{\delta V}{\delta n_1}$=partial molal volume of X_1

$\overline{V}_2=\frac{\delta V}{\delta n_2}$=partial molal volume of X_2

n_1=number of mols of X_1 (water in this case)

n_2=number of mols of X_2 (sucrose in this case).

Obviously $\overline{V}_1$ and $\overline{V}_2$ can represent partial specific volumes if n_1 and n_2 indicate grams of the respective components. The values obtained by this equation agree very closely with those reported in column 5, the agreement depending upon the accuracy with which the partial molal volumes used in these calculations can be evaluated graphically.

TABLE 5.—*Theoretically calculated changes in volume accompanying freezing of sucrose solution* [1]

Weight of solution containing 20 g of sucrose	Weight of ice separated from 100 g of original sclution	Concentration of sucrose in solution	Specific volume of solution	Volume of sucrose solution	Change in volume of solution accompanying removal of water	Volume of ice separated from solution (specific volume= 1.0962)	Volume of 100 g of mixture (solution + ice)	Increase in volume due to formation of ice	Decrease in volume of ice due to lowering of temperature	Decrease in volume of solution due to lowering of temperature	Net expansion due to formation of ice	Net expansion per gram of ice formed
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Grams	*Grams*	*Percent*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*
100	0	20.000	0.92510	92.510	0	0	92.510	0	0	0	0	0
90	10	22.222	.91667	82.500	10.010	10.962	93.462	.952	.0012	.0107	.940	.0940
80	20	25.000	.90616	72.493	20.017	21.924	94.417	1.907	.0036	.0113	1.882	.0941
70	30	28.571	.89270	62.489	30.021	32.886	95.375	2.865	.0086	.0187	2.838	.0944
60	40	33.333	.87483	52.490	40.020	43.848	96.338	3.828	.0166	.0227	3.789	.0948
50	50	40.000	.85002	42.501	50.009	54.810	97.311	4.801	.039	.038	4.724	.0945
40	60	50.000	.81329	32.532	59.978	65.772	98.304	5.794	.074	.058	5.662	.0944
30	70	66.667	.75385	22.616	69.894	76.734	99.350	6.840	.166	.138	6.537	.0934
25	75	80.000	.70836	17.709	74.801	82.215	99.924	7.414	.24	.20	6.97	.093

[1] The calculated values in columns 1 to 9, inclusive, are based on the assumption that temperature remains constant. The values in columns 10 and 11 take into account changes in volume due to changes in temperature necessary to cause the desired freezing, assuming the initial temperature to be the freezing point of the original solution. The last 2 columns give the net changes in volume.

The following equation might also be used for these calculations:

$$dV=\overline{V}_1 dn_1+\overline{V}_2 dn_2 \qquad (2)$$

where

dV=change in volume of the solution

dn_1 and dn_2 refer to the changes in the number of mols of water and sucrose, respectively

$\overline{V}_1$ and $\overline{V}_2$ have the same significance as in equation (1).

It will be recalled that as freezing progresses the amount of sucrose in solution remains constant even though its concentration in the solution increases. On this basis $dn_2=0$. Hence the equation for the particular case becomes

$$dV=\overline{V}_1 dn_1$$

or

$$\Delta V=\int_a^b \overline{V}_1 dn_1 \qquad (3)$$

Evaluating equation (3) graphically gives values almost identical with those of column 6.

Both equations (1) and (2) can be extended to represent more complex systems such, for example, as a solution containing lactose and possibly other dissolved substances in addition to sucrose. Equation (2) is of special interest in this case. In its general form it may be written

$$dV=\overline{V}_1 dn_1+\overline{V}_2 dn_2+\overline{V}_3 dn_3+\overline{V}_4 dn_4 \ldots \qquad (4)$$

If water is the only component of the system which is removed from solution then dn_2, dn_3, dn_4, etc., all become zero and the equation is again reduced to

$$\Delta V=\int_a^b \overline{V}_1 dn_1$$

In other words, we can neglect the partial molal volumes of components other than water if we know that water is the only component separating from solution during the freezing process.

If we consider that the water which is removed is converted into ice without changing the temperature of the system, then the volume of the mixture of ice and solution will be the sum of the volumes of the solution and ice at the original temperature. Ice was considered to have a specific volume of 1.0962 at 20° C. (This value was obtained by using 0.00027 as the thermal coefficient of cubic expansion for ice and extrapolating from 0° to 20°.) The results of these calculations are given in column 8 of table 5 and were obtained by adding the values in columns 5 and 7.

By subtracting from the respective values in column 8 those given in column 5, data are obtained which may be considered to represent the theoretical increases in volume accompanying the formation of ice at constant temperature. Actually, however, the freezing process is

accompanied by lowering of temperature, which in turn causes decreases in volume of both ice and unfrozen solution.

By taking data from figure 3 as a basis for deciding the changes in temperature necessary to remove from solution the required amount of water as ice, and noting the changes in thermal coefficient of expansion of ice covering this range in temperature (*13, v. 3, p. 43*), values have been calculated to show the decrease in volume of ice as compared with its volume at the original temperature. These data are given in column 10. Adequate data are not available for a consideration of the influence of temperature upon the volume of the solution, but by using data for a slightly higher temperature (0° to 20° C.) as reported by Karas (*10*) it has been possible to approximate these changes in volume. The results of these calculations are given in column 11.

Taking into account the values in columns 10 and 11 as a basis for modifying those in column 9, the net increase in volume due to the formation of ice is found, as shown in column 12. The expansion per gram of ice formed is tabulated in column 13. These values deviate only slightly from 0.0944 cc, which is the increase in volume per gram of ice formed at the initial temperature in this study.

The foregoing calculations indicate that the volume changes accompanying freezing are due primarily to the change in state of the water in solution, that is, its separation in the form of ice. The values calculated theoretically also seem to justify the use of a single expansion factor per gram of ice formed over the temperature range considered. Further justification for this assumption is to be found in the agreement between freezing-point data and dilatometer data obtained by using a single expansion factor over the temperature range employed in this study.

DISCUSSION

Attempts to measure the amount of ice formed in ice cream and related products are beset with difficulties, and the use of the dilatometer does not remove them. It does have certain advantages, however, over earlier methods. In standardizing the apparatus, accurate freezing-point data for sucrose solutions were not available for temperatures much below −13° C., yet an attempt was made to measure the amount of ice formed in this work down to −25°. Obviously this check could not be applied to results at the lower temperatures. The data obtained, however, agree with the values that would be expected by extrapolating beyond the values given in the International Critical Tables (*13, v. 4, p. 263*), as shown in figure 3.

If the densities of water and ice are considered at 0° C., the theoretical expansion factor accompanying the change of 1 g of water into ice would be 0.0907 cc. It is difficult to say why the expansion factor obtained with the dilatometer differed from the theoretical expansion factor for water to ice. Air in the system was possibly a contributory factor, since the solubility of air in ice is low as compared to its solubility in the solution at the same temperature. As more of the water from the solution was frozen, it would increase the amount of air set free, thereby giving an expansion factor greater than would otherwise be expected. By setting up a more elaborate means of exhausting the samples of air one could determine whether or not air was a contributing factor. In this connection, however, it may be mentioned that Foote and Saxton (*6*), working with inorganic hydrosols

under somewhat similar conditions, obtained an expansion factor of 0.09325 cc per gram of water changed to ice, as compared with 0.0933 cc used in this study, whereas Bouyoucos (*2*), working with soils, used an expansion factor of 0.10 cc per gram of water frozen.

As already indicated, Leighton (*11*) has suggested a method of approximating the amount of ice formed in ice cream. His calculations are based, among other things, upon the freezing-point depression data for sucrose solutions given by Pickering (*14*). The lowest temperature reported by this author, however, is −13.8° C. Conceivably such calculations might be extended to lower temperatures—for example, −25.0° to −15.0°—as a result of utilizing the data reported in table 1.

It was difficult to initiate freezing while making freezing-point determinations on some of the samples concentrated by the evaporation method. This trouble was presumably caused in part by the high concentration of gelatin and the accompanying increase in viscosity of the samples. For this reason the freezing-point determinations on highly concentrated samples cannot be considered as reliable as those made on the samples before they were concentrated.

In evaluating the partial molal volumes for sucrose and water at various concentrations of sucrose the data used were taken at 20° C. No data from which these values can be calculated are available for the temperature range in these experiments. Such data, if available, might perhaps make a significant difference in the calculated values reported in the section dealing with theoretical considerations. The partial molal volumes of the components other than sucrose and water were not considered.

Zoller (*16*) in 1921 advanced the idea that the percentages of water frozen in a sample of ice cream at a particular temperature would not be equal in all cases. He states (*16, p. 45*):

> The temperature at which the mix is drawn from the machine may be the same (for the same ice cream mixture) in the case of a dozen freezings and yet some may be soft with 15 per cent. of the water frozen while some may be stiff with 40 to 50 per cent. of the water frozen into ice.

Bradley and Dahle (*3*) have recently subscribed to this belief as a basis for explaining differences in texture of ice cream frozen in a continuous (Vogt) freezer as compared with freezing in a batch freezer.

Although such differences would be expected if one or more of the substances dissolved were to crystallize out of solution, it seems unlikely, under commercial conditions, that any significant differences would occur at a given temperature while the mix in question is in the freezer. In the first place, vigorous agitation takes place while the sample is being frozen whether a batch or a continuous-type freezer is employed. In the second place, ice crystals would usually be present in the freezer to initiate freezing as soon as the temperature of the mix had been lowered sufficiently. Although ice would not be present at the beginning of a day's run, it would be present at the beginning of each subsequent run with the batch freezer and continuously after freezing had started in the continuous freezer. As both these factors would tend to prevent supercooling, it might be expected that if different portions of the same mix were frozen in batch and in continuous freezers and withdrawn from these freezers at the same temperature, the percentage of water frozen would be the same in both cases. Conclusive proof of this statement is not available from

the data presented, since the conditions under which freezing occurred in the dilatometer were not comparable in many respects to those under which mixes are frozen commercially. Considerable variation did occur, however, in the rate of freezing of the same sample in this apparatus at different times. In such cases, when equilibrium had been established for a certain temperature, the same amount of ice formed no matter whether the freezing was accomplished rapidly or slowly.

If lactose or any other component present, other than water, were to crystallize out of solution in one trial and not in another, even though portions of the same mix were frozen, then the same percentages of ice would not be formed in the two portions of this sample even if examined at the same temperature. In this study, however, there was no indication that any other component besides water crystallized while the measurements were being made in the dilatometer, although the concentrations of sucrose and lactose were greater in the experimental samples than would be permitted by saturated solutions at these low temperatures. A given sample refrozen several times formed practically the same percentages of ice in successive determinations whether the freezing or the melting curves were considered.

The trend in commercial practice has definitely been toward fast-frozen ice creams. Most of the new freezers are designed to permit rapid heat transfer. Some are so constructed that the ice cream can be regularly withdrawn from the freezer at −6.0° to −5.5° C. (21.2° to 22.1° F.), which for a sample like M–8 would mean that 55 to 48 percent of the water had been frozen. The common practice, where batch freezers have been used, has been to draw ice cream at from −4.0° to −3.3° C. (24.8° to 26.1° F.), which would show from 36 to 24 percent of the water frozen for the sample M–8. The advantage of rapidly freezing as much of the water as possible has been indicated previously (*3, 4, 5*), although many manufacturers still overlook the benefits obtainable by this practice.

Certainly the installation of air-circulating systems in hardening rooms as well as blast tunnels, and the use of relatively low temperatures, are desirable practices as regards the improvement of ice-cream texture. Even here, however, the rate of freezing is slow as compared with the rate at which ice is formed in the freezer.

The data in this paper should emphasize the differences in the amounts of ice formed at various temperatures. These differences, if correlated with the rate at which freezing occurs in practice, may help to determine the effects of such variations upon the quality of the finished products.

SUMMARY

The use of the dilatometer method as a basis for measuring the amount of ice formed in ice cream and related products has been described.

By this method, measurements were made on nine ice-cream, three ice-milk, and four water-ice samples. The results presented show the percentages of water frozen in various mixes over a temperature range from about −25° to 0° C.

On the basis of the observations with sucrose solutions, data are presented to show the relation between freezing-point lowering and

concentration at temperatures below that given by Pickering (*14*) or reported in the International Critical Tables (*13*). A suggestion is made as to how these additional data can be used in calculating the amount of ice formed in ice cream.

The results given may serve as a basis for improving commercial practices in freezing such products as ice cream, since quality is influenced by the amount of ice frozen at certain stages of manufacture and distribution.

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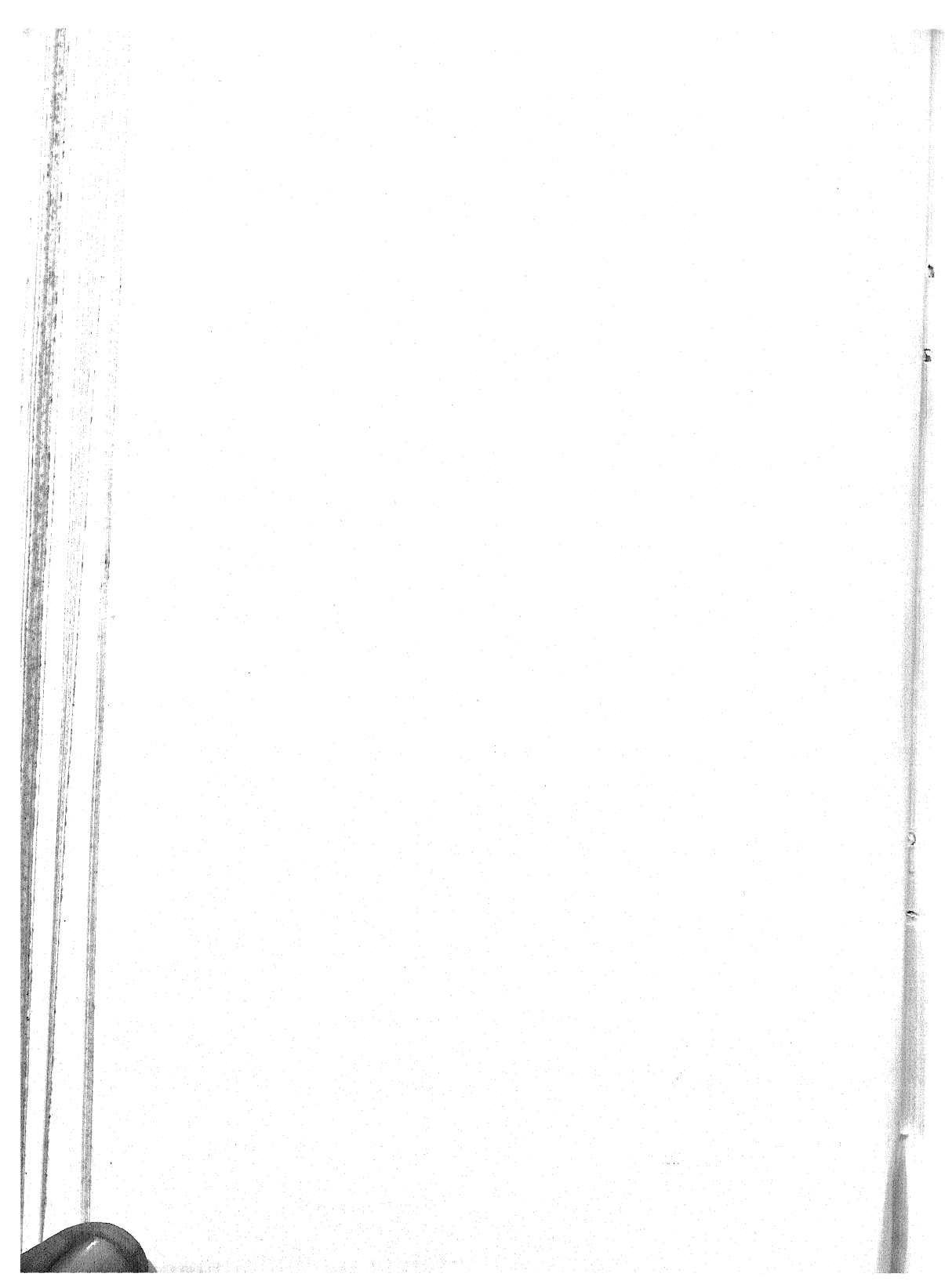
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A METHOD FOR EXTRACTING GROWTH SUBSTANCES FROM PIGMENTED TISSUES [1]

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That natural growth substances in green plants occur in different concentrations has not yet been actually proved, but there are many indications that such is the case. Two methods to demonstrate the presence and concentration of growth regulators are in use: (1) The diffusion method (Stark (*10*),[3] Seubert (*8*), and Went (*12*)), and (2) the extraction method (Nielsen (*7*), Kögl, Erxleben, and Haagen-Smit (*5*), and Thimann (*11*)) and others.

The diffusion method has been used lately by many investigators (e. g., Avery, Burkholder, and Creighton (*2*) to demonstrate the regulatory effect of stem growth; Delisle, 1937, (*3*) in his investigation on leaf development, etc.). In some cases the results differ from those obtained by the extraction method on "colorless" plants. The differences may be accounted for by the fact that the diffusion method gives information only on the transport of the substances, and not on the actual concentration. If the transport is low, the concentration may be very high, and vice versa (cf. Söding (*9*, *p. 841*)).

The above-mentioned extraction methods have been used for the lower plants and for etiolated higher plants. When green plants have been extracted very low concentrations have been found, or the curvatures were irregular, or, as many investigators have noted, the curvatures were positive. Avery's extractions of *Nicotiana* leaves (*1*) gave only relative values, as can be seen by a comparison of his values with those obtained by diffusion. Jahnel (*4*) obtained very variable results from extractions of potato, except in one case.

The extraction method described below permits a more precise determination of the actual amount of growth substances in pigmented plants than is possible by the methods discussed above. A trial of the other methods indicated that the main trouble begins when chlorophyll, or, in the case of potatoes, some strongly adsorbing or oxidizing substance is extracted with the auxin. To obviate this difficulty the tissue is frozen with solid carbon dioxide. The carbon dioxide atmosphere prevents the activity of oxidizing agents, and the freezing makes the tissue very brittle and permits a thorough grinding. After the grinding, an excess of slightly acidified water is added which thaws the tissue slowly and in the meantime dissolves the auxins, apparently before other substances of the extract can destroy them. Before the temperature has risen the mixture is filtered through a Büchner filter and washed out at least twice with water at a temperature of about 0° C. The filtrate is then washed three times with ether or chloroform. These solvents are each time separated from

[1] Received for publication July 28, 1937; issued March, 1938.

[2] The writer wishes to express his thanks to Dr. N. L. Drake for helpful suggestions in the course of the work.

[3] Reference is made by number (italic) to Literature Cited, p. 157.

the water layer and contain most of the growth substance. The solvent is then evaporated until about 0.1 cc is left. The evaporation must be done very slowly. It is even advisable to collect the ether or the chloroform and evaporate it again and to add the two residues for further treatment; without this precaution negative results are frequent. Two-tenths of a cubic centimeter of 2 percent or 3 percent agar is added. The solvent is then further evaporated. The 0.2 cc of agar with the auxin is put into an agar divider [4] (fig. 1), which consists of a thin plate (*a*), the aperture of which provides, when applied to the base of the cutter, a container of 0.2-cc capacity; four small knives (made from safety razor blades) in holder are guided by a pin (*b*) on the vertical axis which slides in a groove. By lifting the holder (aided by a spring) it can be turned 90° and so allow the pin to slide down along a second groove (*d*). The razor blades make second cuts in the agar perpendicular to the first ones. Thus the agar is divided into 25 equal blocks of 8 mm³, providing enough material for two series of 12 plants each, with practically no loss of growth substance.

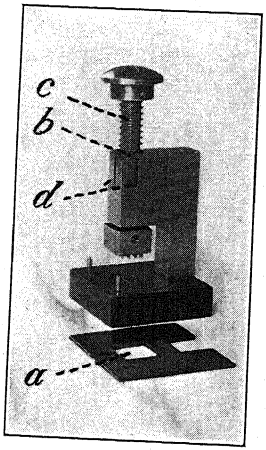


FIGURE 1.—The agar divider: *a*, Container; *b*, pin; *c*, vertical axis; *d*, groove.

In many of the tests a strongly emulsifying agent passed through the filter. In those cases the ether or chloroform layer was centrifuged and the jellylike mass which separated on top of the clear liquid and which contained some growth substances was added to the watery layer and washed out with it.

An effort was made to remove the emulsifying agents with lead salts, but they did not precipitate out. An attempt was then made to adsorb the emulsifying agents on active carbons of different quality by adding carbon to the watery solution, to the filter paper in the Büchner funnel before use, or to the filtrate. In all cases, however, the nontreated portion of the solution appeared to contain more of the growth regulators, which means that the growth substance was partially adsorbed by the carbon. Laibach and Lotz (*6*) state that if their procedure is precisely adhered to, the growth substance is adsorbed slightly or not at all on active carbon. Their table, however, shows about 50 percent adsorption for the first solution, if their fourth solution means a solution that is diluted four times. Moreover, the chemical structure (polar groups) of the true auxins shows that an adsorption on active agents is very likely to occur. The adsorption method can be applied directly to the material ground with solid carbon dioxide.

Some results obtained by the method described above are given in table 1. Extractions by other methods of the plants mentioned in table 1 gave irregular results (either small and variable or positive curvatures).

[4] This divider was constructed for the writer by P. A. de Bouter (Utrecht), and a later one by O. Westgard (Cambridge, Mass.).

TABLE 1.—*Concentration of growth substance obtained from different plant sources by different methods of extraction*

Source of extract concentrated in 25 agar blocks	Weight of material used	Method of extraction	Curvature of oat seedlings per agar block applied
	Grams		*Degrees*
Wheat seeds, soft dough	20	Solid carbon dioxide	22.9±1.5
6 immature heads of wheat plants	27	do	20.1±1.6
Basal parts of wheat plants	15	do	17.1± .2
10 tips of bryophyllum plants		do	19.3±1.3
1 tip of bryophyllum per agar block for 1 hour.		Diffusion	17.0±1.1
Tissue of potato tuber underneath buds	8	Solid carbon dioxide	19.3±1.7
Thallus of fucus		do	21.9±2.9
		Chloroform	0
35 oat seedlings		do	[1] 6.1± .9
		Solid carbon dioxide	[1] 28.8±1.9
		Solid carbon dioxide, carbon on filter	[1] 7.9±1.4
Primary leaves of oat seedlings	2.5	Solid carbon dioxide, carbon in solution	[1] 2
		Solid carbon dioxide	[1] 5.2± .5

[1] Tested simultaneously.

SUMMARY

A method for extracting growth regulators from pigmented plants is described. This method permits the demonstration of growth substances in plants that have given irregular results when extracted by other methods.

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INHERITANCE OF RESISTANCE TO LOOSE AND COVERED SMUTS IN MARKTON OAT HYBRIDS[1]

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INTRODUCTION

Inheritance of smut resistance in oat hybrids has attracted the attention of an increasing number of investigators. The literature has been reviewed by Coffman et al. (*2*)[2] Reed (*6*), and Johnson (*4*). Since these reviews were published other papers have appeared by Nicolaisen (*5*), Schattenberg (*9*), Stanton, Coffman, and Tapke (*10*), Stanton, Reed, and Coffman (*11*), Reed (*7, 8*), and Austin and Robertson (*1*).

The present paper is concerned with the investigation of the inheritance of resistance to loose smut (*Ustilago avenae*) (Pers. Jens.) and covered smut (*U. levis* (Kell. and Sw.) Magn.) in oat hybrids involving the smut-resistant Markton and five other parental varieties showing contrasting reactions to these smuts.

MATERIAL AND METHODS

The strains of the varieties used in these hybrids belong to *Avena sativa* L. and have been grown by the senior author for many years in connection with his investigations of the oat smuts. With the exception of Markton, all these type varieties have been described by Etheridge (*3*). Information on the origin and characters of Markton is given in other publications (*2, 10, 11*). The hybrids were made primarily for studies of the mode of inheritance of smut resistance and not for the development of resistant varieties for commercial production. In all hybrid combinations the female parent is named first. On the basis of the smut reaction of these varieties the different hybrids may be grouped as follows:

Group 1.—One parental variety resistant and the other susceptible to both loose and covered smut:
Hybrid 50, Canadian × Markton.
Hybrid 51, Early Champion × Markton.
Hybrid 52, Markton × Early Champion.
Hybrid 53, Victor × Markton.

Group 2.—One parental variety resistant to both smuts and the other susceptible to loose smut but resistant to covered smut:
Hybrid 56, Gothland × Markton.

Group 3.—One parental variety resistant to both smuts and the other susceptible to covered smut, but resistant to loose smut, the direct opposite or antithesis of hybrid 56:
Hybrid 60, Monarch × Markton.

The hybrids were made in 1928 at Aberdeen, Idaho, by Clem Ault, under the direction of G. A. Wiebe, who at that time was assistant

[1] Received for publication May 21, 1937; issued March 1938. The investigations herein reported were conducted cooperatively by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Brooklyn Botanic Garden, Brooklyn, N. Y. Brooklyn Botanic Garden Contribution No. 80.

[2] Reference is made by number (italic) to Literature Cited, p. 176.

agronomist in charge of the cooperative cereal experiments at the Aberdeen substation. Seed of the special strains was furnished by the senior author and forwarded to Aberdeen by the junior author for sowing.

The F_1 plants were grown at the Brooklyn Botanic Garden in 1929, and the F_2, F_3, and F_4 in subsequent years. The method of inoculation employed was developed by the senior author in the course of his investigations with the oat smuts. After the removal of the hulls, the caryopses were inoculated with the dry spores of *Ustilago avenae*-Missouri or *U. levis*-Missouri as described. The seeds were germinated at a temperature of 20° C. in sand containing about 20 percent moisture. The seedlings usually emerged in 4 days and were large enough to transplant 2 to 3 days later.

In the case of all hybrids, three series of F_2 plants were grown: (1) Uninoculated, (2) inoculated with loose smut, and (3) inoculated with covered smut. Consequently, in growing the F_3 generations, there were three groups of progenies available: (1) Those descended from uninoculated F_2 plants, (2) those descended from F_2 plants inoculated with loose smut, and (3) those descended from F_2 plants inoculated with covered smut.

EXPERIMENTAL DATA

REACTION OF PARENTAL VARIETIES

The reaction of the parental varieties to the Missouri races of loose smut and covered smut was determined. During the period from 1930 to 1934, populations of the parents were grown along with the various hybrid generations, and all the data for the parental varieties and data obtained in 1930 on the reactions of the F_2 plants are summarized in table 1.

TABLE 1.—*Reaction to the Missouri races of loose and covered smut of inoculated F_2 plants of 5 oat hybrid combinations and their parental varieties grown at the Brooklyn Botanic Garden, 1930* [1]

Group, parental variety, and hybrid	Hybrid no.	Reaction to *Ustilago avenae*			Reaction to *Ustilago levis*		
		Plants grown	Plants infected		Plants grown	Plants infected	
		Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Group 1:							
Canadian (119) [2]		319	316	99.1	373	370	99.2
Canadian × Markton	50	81	7	8.6	80	26	32.5
Markton (752)		681	9	1.3	763	7	.9
Early Champion (150)		320	290	90.6	314	267	85.0
Early Champion × Markton	51	230	26	11.3	227	66	29.1
Markton × Early Champion	52	75	12	16.0	20	4	20.0
Victor (126)		509	488	95.9	690	656	95.1
Victor × Markton	53	182	22	12.1	130	30	23.1
Group 2:							
Gothland (152)		672	645	96.0	751	0	0
Gothland × Markton	56	170	29	17.1	161	9	5.6
Group 3:							
Monarch (161)		1,079	4	.4	1,078	1,036	96.1
Monarch × Markton	60	158	2	1.3	246	54	22.0

[1] All data for parental varieties for 1930–34 included.

[2] Numbers in parentheses indicate special seed numbers used by G. M. Reed to designate his particular experimental strains of these varietal types.

The parent varieties Canadian, Early Champion, Gothland, and Victor gave from 90.6 to 99.1 percent infection with loose smut. Only 4 of 1,079 plants grown of the resistant Monarch were infected with *Ustilago avenae*. Canadian, Early Champion, Monarch, and Victor were very susceptible to covered smut, giving from 85 to 99.2 percent infection. No smutted plants appeared in the resistant Gothland.

Markton showed a very high degree of resistance to both loose and covered smuts. Most of the populations grown contained no smutted individuals. Occasionally, however, a smutted plant was observed. This occurred somewhat more frequently with loose smut than with covered smut.

Among the plants grown from inoculated seed of Markton, a few were observed that were described as blasted.[3] Such plants usually produced infertile spikelets. When these plants were first noted, the sterile condition was not attributed to possible infection by the smut organism. The fact, however, that such types of panicles appeared rather commonly among the hybrids of which Markton was one of the parents suggested that this sterility was really due to the infection of the plant by the smut. A study of these plants revealed the fact that various degrees of sterility appeared in the panicles. In some cases the spikelets were mere rudiments (fig. 1); in others they were better developed and frequently contained a small number of smut spores (fig. 2). Typically smutted panicles (fig. 3) were observed in the same rows.

This peculiar behavior of Markton toward the smuts must be considered in the study of the inheritance of resistance. For the most part plants in which smut spores could not be readily observed were not counted as infected. This was especially true in the earlier phases of the investigation. If this type of infected plant had been included in recording the data, the percentage of smut in the F_2, F_3, and later generations would be somewhat higher.

REACTION OF THE F_2 GENERATION

The data obtained on the F_2 generation for all six crosses are recorded in table 1.

While Canadian has shown equally high susceptibility to both smuts, in the Canadian × Markton combination (hybrid 50) a much higher percentage of the plants of the hybrids were infected with covered smut than with loose smut.

In hybrid 51, in which Early Champion was used as the female parent and Markton as the male, and in hybrid 52, its reciprocal, it will be noted from table 1 that considerably higher percentages were obtained with covered smut than with loose smut and that there was some variation in the reaction in F_2 of the populations from the initial and reciprocal crosses.

In hybrid 53, Victor × Markton, a much higher percentage of infection also was obtained with the covered smut, although the Victor parent was almost 100 percent infected with both smuts.

[3] A relatively small percentage of similar sterile plants were observed as early as 1925 in F_3 populations of Markton × Idamine, Markton × Victory, etc., at the Aberdeen substation, by T. R. Stanton, F. A. Coffman, and G. A. Wiebe. While no systemic examination was made at the time, it was believed that the blasted appearance of the plants resulted from a form of sterility probably caused by smut infection.

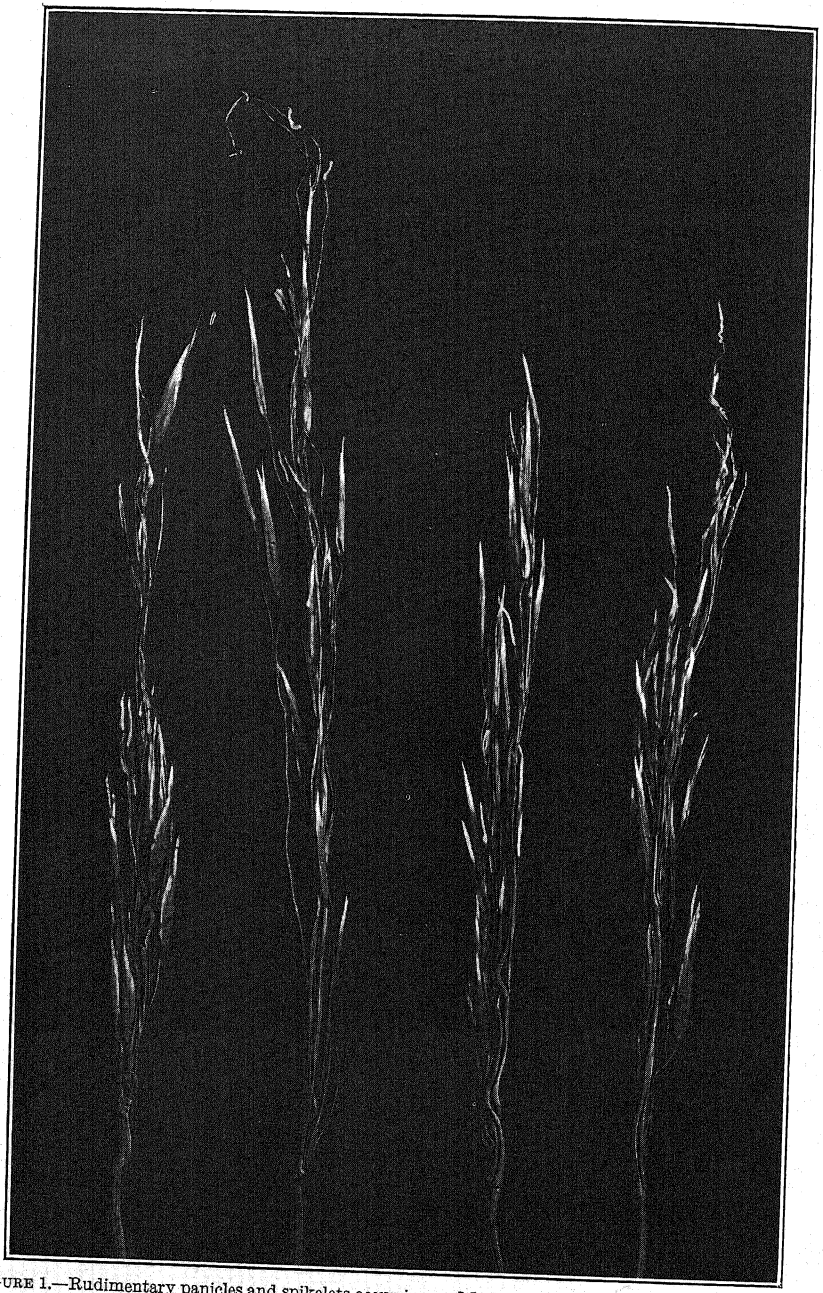


FIGURE 1.—Rudimentary panicles and spikelets occurring on Markton hybrid 50-c-F-229-2, showing characteristic sterility and no external evidence of smut pustules.

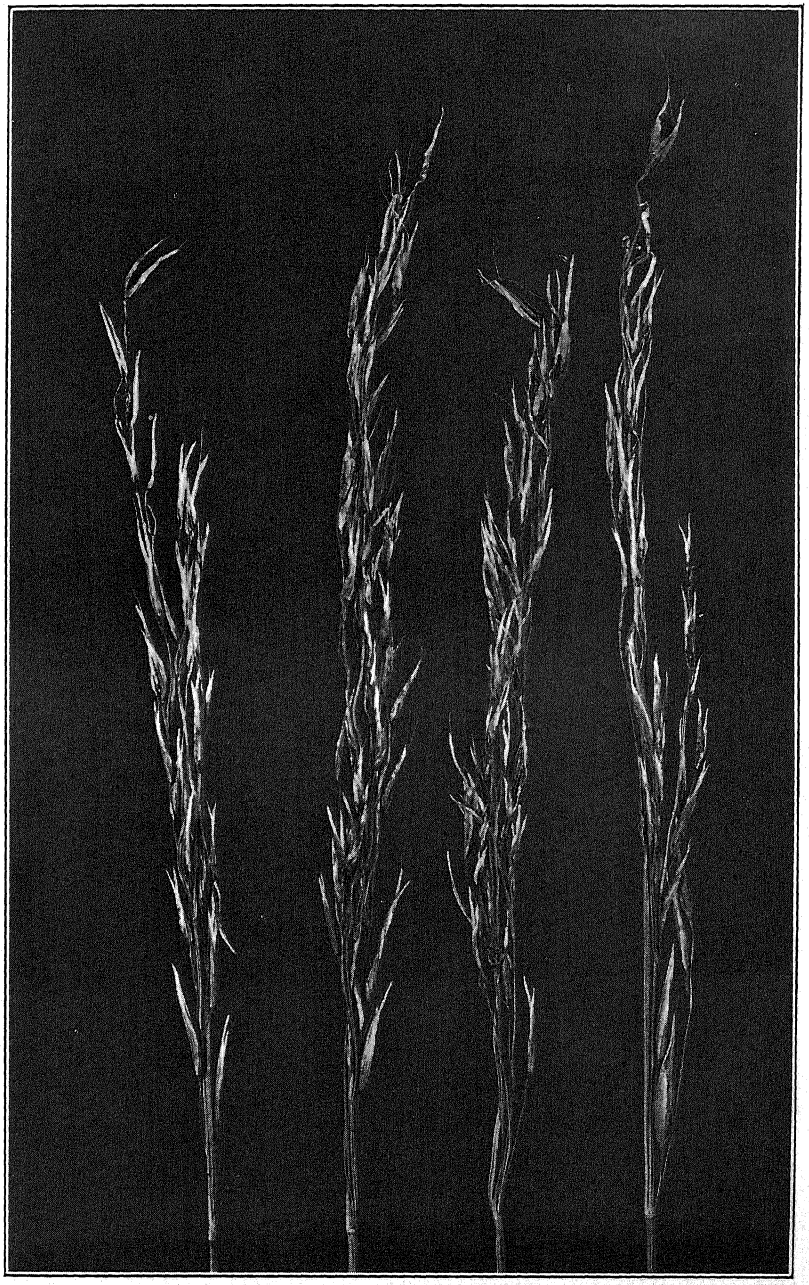


FIGURE 2.—Rudimentary panicles and spikelets occurring on Markton hybrid 51-F_4-223-3, representing a second type of sterility with some smut pustules but on undersized kernels.



FIGURE 3.—Typical development of *Ustilago levis* on Markton hybrid 51-F_4-223-3.

Hybrid 56, Gothland × Markton, represents the second group of oat hybrids included in this study, in which one parent, Markton, is resistant to both smuts, while the other parent, Gothland, is susceptible to the loose smut but resistant to the covered. It is interesting to note that a considerable number of F_2 plants were infected by covered smut, although both Gothland and Markton, as shown by the data in table 1, are extremely resistant.

Hybrid 60, Monarch × Markton, represents the third group of hybrids, in which a variety (Markton) resistant to both smuts is crossed with another variety (Monarch) susceptible to the covered smut but resistant to the loose smut. In this combination Monarch was used as the female parent. The average infection for all F_2 plants was 22 percent for covered smut. Of the F_2 plants inoculated with loose smut, 1.3 percent were unexpectedly infected.

The behavior of hybrid 60 in the loose smut experiments is similar to that of hybrid 56 in the covered smut experiments. The appearance of plants infected with *Ustilago avenae* in F_2 populations of hybrid 60, in which both parents have shown virtually 100 percent resistance, is of much genetic interest. In hybrid 56 the appearance of 5.6 percent of smutted plants in the *U. levis* series with both parents 100-percent resistant is likewise unusual.

REACTION OF THE F_3 GENERATION

A summary of data on the reaction of the F_3 progenies of the different hybrids is given in table 2. The progenies are grouped in 11 classes on the basis of class centers for percentages of smutted plants. On the basis of behavior, the F_3 progenies are grouped empirically as resistant (no infected plants observed), segregating (50 percent infection or less), and susceptible (more than 50 percent infection).

TABLE 2.—*Distribution of F_3 progenies of hybrids between Markton and 5 varieties of oats, based on the percentage of plants infected with Ustilago avenae and U. levis*

REACTION TO U. AVENAE

Smut used for inoculation in F_2 and hybrid	Hybrid no.	Infection in F_2 [1]	F_3 progenies in indicated class center for percentage of infection [2]													Total F_3 progenies	F_3 progenies infected
			Resistant	Segregating						Susceptible							
			0	5	15	25	35	45	Total	55	65	75	85	100	Total		
		Percent	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Not inoculated:																	
Canadian × Markton	50		25	14	10	2	4	2	32	1	0	0	0	0	1	58	56.9
Early Champion × Markton [3]	51		25	18	10	7	6	0	41	1	0	2	1	1	5	71	64.8
Victor × Markton	53		19	12	10	2	3	2	29	0	0	0	1	0	1	49	61.2
Gothland × Markton	56		23	29	17	7	5	3	61	0	0	0	0	0	0	84	72.6
Monarch × Markton	60		19	7	1	1	0	0	9	0	0	0	0	0	0	28	32.1
U. avenae:																	
Canadian × Markton	50	8.6	5	9	3	1	0	0	13	0	0	0	0	0	0	18	72.2
Early Champion × Markton	51	12.5	30	21	9	5	3	0	38	0	0	0	0	0	0	68	55.9
Victor × Markton	53	12.1	24	11	10	1	0	0	22	0	1	0	0	0	[4] 1	47	48.9
Gothland × Markton	56	17.1	22	16	5	1	1	0	23	1	0	0	0	0	[4] 1	46	52.2
Monarch × Markton	60	1.3	18	1	2	1	0	0	4	0	0	0	0	0	0	22	18.2
U. levis:																	
Canadian × Markton	50	32.5	5	4	3	2	2	1	12	1	0	0	0	0	1	18	72.2
Early Champion × Markton [3]	51	28.3	24	22	6	3	1	0	32	0	1	0	0	0	1	57	57.9
Victor × Markton	53	23.1	19	5	7	2	0	0	14	0	0	0	1	0	1	34	44.1
Gothland × Markton	56	5.6	14	17	9	2	1	0	29	1	0	0	0	0	1	44	68.2
Monarch × Markton	60	22.0	27	1	1	0	0	0	2	0	0	0	0	0	0	29	6.9

REACTION TO U. LEVIS

Not inoculated:																	
Canadian × Markton	50	-------	21	17	11	4	3	1	36	1	0	0	0	0	1	58	63.8
Early Champion × Markton [3]	51	-------	16	21	11	5	9	3	49	3	3	0	1	0	7	72	77.8
Victor × Markton	53	-------	19	13	5	7	4	2	31	1	0	0	0	0	1	51	62.7
Gothland × Markton	56	-------	22	6	1	1	0	0	8	0	0	0	0	0	0	30	26.7
Monarch × Markton	60	-------	31	33	19	11	7	10	80	6	4	0	0	0	10	121	74.4
U. avenae:																	
Canadian × Markton	50	8.6	9	3	4	0	2	0	9	0	0	0	0	0	0	18	50.0
Early Champion × Markton [3]	51	12.5	28	26	5	5	3	0	39	1	0	0	0	0	1	68	58.8
Victor × Markton	53	12.1	25	11	6	1	2	1	21	0	1	0	0	0	1	47	46.8
Gothland × Markton	56	17.1	35	8	1	0	1	0	10	1	0	0	0	0	1	46	23.9
Monarch × Markton	60	1.3	6	5	1	4	3	2	15	0	0	1	0	0	1	22	72.7
U. levis:																	
Canadian × Markton	50	32.5	6	6	2	2	1	0	11	1	0	0	0	0	[4] 1	18	66.7
Early Champion × Markton [3]	51	28.3	27	17	6	4	2	1	30	0	0	0	0	0	0	57	52.6
Victor × Markton	53	23.1	20	8	3	2	0	1	14	0	0	0	0	0	0	34	41.2
Gothland × Markton	56	5.6	29	6	5	3	1	0	15	0	0	0	0	0	0	44	34.1
Monarch × Markton	60	22.0	9	9	6	4	0	1	20	0	0	0	0	0	0	29	69.0

[1] Smut infection obtained in F_2 as indicated in table 1.
[2] 0=resistant; 1–50=segregating; 51–100=susceptible.
[3] Including data for the reciprocal (hybrid 52).
[4] May represent an escape in F_2.

Before F_2 results are compared with those obtained for F_3 it should be stated that, insofar as it has been possible to determine, no marked difference in the behavior of the F_3 progenies of hybrids 51 (Markton × Early Champion) and 52 (Early Champion × Markton) has been observed. Accordingly, the results from the two hybrids have been combined for study.

In general, the chief genetic interest is found in the data for the F_2 generation and for the first group of F_3 progenies, that is, those descended from uninoculated F_2 plants. All three types of progenies, resistant, segregating, and susceptible, may be expected, since no susceptible F_2 plants have been eliminated. The uninoculated group is mainly considered in the paragraphs that follow; brief references also are made to the reactions of the other two groups of progenies, namely, those descended from F_2 plants inoculated with loose smut and covered smut.

INTERPRETATION OF THE REACTION OF THE HYBRIDS TO USTILAGO AVENAE

Hybrid 50, Canadian × Markton.—Of the 81 F_2 plants inoculated with *Ustilago avenae*, 8.6 percent were infected. In the F_3, 56.9 percent of the progenies descended from uninoculated F_2 plants contained smutted plants. The data for both the F_2 and F_3 generations suggest a possible two-factor relation. If two independent factors for resistance are concerned, 6.25 percent of the F_2 plants should be infected and 56.5 percent of the F_3 progenies should contain smutted plants, very few of which, however, would be classified as susceptible.

Hybrids 51 and 52, Early Champion × Markton and the reciprocal.—Altogether, 305 F_2 plants of the two hybrids were inoculated, of which 38, or 12.5 percent, were infected. In all, 64.8 percent of the F_3 progenies descended from uninoculated F_2 plants contained smutted individuals. These results may suggest a single-factor interpretation, although the number of infected F_2 plants was only half of the expectation. Further, about 75 percent of the F_3 progenies would be expected to contain smutted plants, and approximately one-third of these should be highly susceptible.

Hybrid 53, Victor × Markton.—In the F_2 generation of this cross 12.1 percent of the plants were infected and 61.2 percent of the F_3 progenies descended from uninoculated F_2 plants contained smutted individuals. These results likewise indicate a monogenic type of inheritance for loose smut.

Hybrid 56, Gothland × Markton.—Of this cross 17.1 percent of the F_2 plants were infected. No susceptible F_3 progenies descended from uninoculated, F_2 plants were recorded, although 72.6 percent of these progenies contained smutted individuals. The results with both the F_2 and F_3 generations may suggest a single-factor difference, but the complete lack of susceptible F_3 progenies does not conform with this interpretation.

Hybrid 60, Monarch × Markton.—Since both parental varieties are resistant to the loose smut, it is of special interest that 2 F_2 plants out of a total of 158 grown (1.3 percent) were infected. Further, out of the 28 F_3 progenies grown from uninoculated F_2 plants, 9 contained a few infected individuals, the remaining 19 being fully

resistant. The results for both generations may suggest a three-factor difference.

The reaction to *Ustilago avenae* of the other two groups of F_3 progenies descended from F_2 plants inoculated with *U. avenae* and *U. levis*, respectively, may be briefly considered. Since the second group of F_3 progenies was descended from F_2 plants inoculated with loose smut, fully susceptible progenies would be absent unless a susceptible F_2 plant escaped infection. The reaction of the F_3 progenies of this group is summarized in table 2.

Lack of space forbids the presentation of the data in sufficient detail to bring out possible correlations in the behavior of the different hybrids to both smuts, yet there is no evidence of a similarity in the reaction of the F_3 progenies to the two pathogens. All the data obtained indicate a complete independence in their behavior to either smut. Thus, an F_2 plant might be resistant to one of the smuts and resistant, heterozygous, or susceptible to the other. These facts must be kept fully in mind when considering the data obtained with the loose smut for the F_3 progenies (third group) descended from F_2 plants inoculated with covered smut. A summary of the behavior of these progenies is given in table 2.

INTERPRETATION OF THE REACTION OF THE HYBRIDS TO USTILAGO LEVIS

Hybrid 50, Canadian × *Markton.*—Of the 80 F_2 plants inoculated with *Ustilago levis*, 32.5 percent were infected. Twenty-one F_3 progenies descended from uninoculated F_2 plants were classed as resistant, 36 as segregating, and 1 as susceptible, the latter giving 54.1 percent infection. The data for the two generations do not agree very well. Results with the F_2 plants might suggest a single-factor difference. In this case, however, many more F_3 progenies would be expected to contain infected plants, and approximately one-fourth of them should be highly susceptible.

Hybrids 51 and 52, Early Champion × *Markton.*—There were grown 247 F_2 plants, of which 28.3 percent became infected when inoculated with *Ustilago levis*. Of 72 F_3 progenies descended from uninoculated F_2 plants 16, were classed as resistant, 49 as segregating, and 7 as susceptible, the highest percentage of infection obtained being 88.2 percent. In these hybrids also the F_2 data may indicate a single-factor difference. The data for the F_3 generation likewise are in harmony with this interpretation, except that too few highly susceptible progenies were obtained.

Hybrid 53, Victor × *Markton.*—In the F_2 generation 23.1 percent of the plants became infected when inoculated with *Ustilago levis*. Of 51 F_3 progenies, descended from uninoculated F_2 plants, 19 were classed as resistant, 31 as segregating, and 1 as susceptible, the latter giving 58.3 percent infection. Results with this hybrid correspond rather closely with those obtained for hybrid 50. The F_2 data might indicate a single-factor difference. The data for the F_3 generation, however, are not altogether in agreement with this interpretation, as there are too many resistant progenies and a decided lack of highly susceptible ones.

Hybrid 56, Gothland × *Markton.*—Since Gothland and Markton are both extremely resistant to covered smut, no infection among the F_2

plants would be expected. Actually, however, 9 out of 161, or 5.6 percent, were smutted. In the F_3 generation descended from uninoculated F_2 plants, 30 progenies were grown, of which 22 were classed as resistant and 8 as segregating, all giving a low percentage of infection. The results for this hybrid with covered smut are similar to those obtained for hybrid 60 with loose smut.

Hybrid 60, Monarch × Markton.—There were 246 F_2 plants inoculated, of which 54, or 22 percent, were infected. Of 121 F_3 progenies grown from uninoculated F_2 plants, 31 were classed as resistant, 80 as segregating, and 10 as susceptible, infection in the latter ranging from 50.1 to 66.6 percent. The data for both F_2 and F_3 generations may suggest a one-factor difference in this particular cross. Again, however, there is a marked shortage of susceptible F_3 progenies.

F_3 progenies were grown from F_2 plants belonging to each of the other two groups, inoculated with *Ustilago avenae* and *U. levis*, respectively. The reactions to *U. levis* of these F_3 progenies are summarized in table 2.

In both smut series there was a marked shortage of susceptible progenies and in all hybrids there was a strong preponderance of resistant ones.

Hybrid 56 (Gothland × Markton) and hybrid 60 (Monarch × Markton) afford a number of interesting contrasts. In hybrid 56 one of the parents is resistant to covered smut and susceptible to loose smut, while in hybrid 60 the opposite relation obtains.

The reaction of hybrid 56 to loose smut fits fairly well a simple factorial relation, as 17 percent of the F_2 plants were infected when inoculated with *Ustilago avenae*, and approximately 75 percent of the F_3 progenies contained smutted individuals that were descended from uninoculated F_2 plants. Among the latter, however, fully susceptible progenies were entirely lacking. In the covered smut series, 5.6 percent of the F_2 plants were infected and a small number of F_3 progenies contained smutted individuals. Thus, although the two parents were highly resistant, some infected individuals occurred in both the F_2 and F_3 generations. This may be explained on the basis of three factors, one introduced by one parent and two by the other.

Hybrid 60 has shown a reverse reaction. In the covered smut series, 22 percent of the F_2 plants were infected, and resistant, segregating, and susceptible progenies descended from uninoculated F_2 plants were observed in the F_3 generation. The results suggested a single-factor relation, although there was a shortage of susceptible progenies. In the loose smut series only 1.3 percent of the F_2 plants were infected, and a small number of F_3 progenies descended from uninoculated F_2 plants contained a few smutted individuals.

REACTION OF THE F_4 GENERATION

A large number of F_4 families have been grown in the course of the experiments. These were descended from F_3 progenies of which the reaction to both loose and covered smuts was known. Most of the F_4 families were descended from resistant F_3 progenies. It is impossible to consider here all the data obtained, but some of the more significant results are summarized in table 3.

TABLE 3.—*Summary of data on F_4 progenies of hybrids between Markton and five varieties of oats*

[All F_4 families are descended from F_3 progenies resistant to both *Ustilago avenae* and *U. levis*, except as noted]

Hybrid No.	F_3 progenies	F_4 progenies having indicated reaction to—					
		Ustilago avenae			*Ustilago levis*		
		Resistant	Infected	Range of infection	Resistant	Infected	Range of infection
	Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
DESCENDED FROM UNINOCULATED F_2 PLANTS							
50	4	21	0	----------	21	0	----------
51	8	52	9	5.2–29.4	57	4	5.0–35.0
53	7	46	0	----------	45	1	16.6
56	[1] 8	46	4	5.0–25.0	[2] 18	2	5.5– 5.8
60	[1] 4	27	0	----------	[3] 61	3	5.5– 6.6
DESCENDED FROM F_2 PLANTS INOCULATED WITH U. AVENAE							
50	5	13	2	8.3–100	14	1	46.6
51	16	35	13	5.2–53.3	33	15	4.1–16.6
53	14	35	7	5.2–43.7	34	8	4.7–28.0
DESCENDED FROM F_2 PLANTS INOCULATED WITH U. LEVIS							
50	4	10	2	6.2– 6.6	12	0	----------
51	14	31	11	4.3–28.5	38	4	5.0–33.3
53	11	26	6	4.7– 7.6	25	7	4.1–10.3

[1] Resistant to *U. avenae*.
[2] Descended from 4 F_3 progenies resistant to *U. levis*.
[3] Descended from 16 F_3 progenies resistant to *U. levis*.

Most of the F_4 families were completely resistant. In several cases, however, a single plant was infected. A few families gave a somewhat higher percentage of infection. In the F_4 only two families of the total number grown were classified as susceptible, one of these giving 53.3 percent and the other 100 percent infection. These were both in the loose smut series. It is quite possible that in some cases a retest of the F_3 progeny would show that it was actually segregating instead of resistant.

DISCUSSION

Hybrids 50, 51, 52, and 53 involve crosses in which one parent is susceptible to both loose and covered smuts, while the other parent, Markton, is resistant to both. As noted above, all the data obtained indicate a complete independence in the reaction of these hybirds to loose and covered smuts. Accordingly, a progeny resistant to one smut may be resistant, segregating, or susceptible to the other smut.

In all these hybirds there were low percentages (8.6 to 16.0) of F_2 plants infected with the loose smut. On the other hand, relatively high percentages of infection (23.1 in hybrid 53 to 32.5 in hybrid 50) were obtained with covered smut.

In both series of F_3 progenies there were very few susceptible to either loose or covered smut. A failure to obtain susceptible progenies

in the groups descended from uninoculated F_2 plants probably is not due in any way to the methods employed, since, in the parallel series, the susceptible parental varieties regularly gave very high percentages of infection.

The data do not fit well any definite factorial ratio for the inheritance of smut resistance. It may be that the inheritance of resistance to loose smut depends primarily on two main factors. On the other hand, a single-factor difference may account for the results obtained on the inheritance of covered smut.

Several investigators have studied inheritance of resistance in hybrids in which the Markton oat has been used as one parent. Stanton, Reed, and Coffman (*11*) have reported results with Markton × Black Mesdag, Markton × Iogold, Cornellian × Markton, and Richland × Markton. The F_2 and F_3 generations were inoculated with the same races of loose and covered smuts used in the present investigations, and the same methods were employed. Markton and Black Mesdag proved to be very resistant to loose smut in all experiments. In hybrid populations resulting from crossing these varieties, however, one infected F_2 plant was observed, and in the F_3 generation a few progenies contained a small number of infected plants. Iogold and Richland as parents produced rather low percentages of infection with the loose smut, and, when crossed on Markton, a few infected F_2 plants were observed. Several F_3 progenies with smutted individuals also were recorded. Cornellian is moderately susceptible to loose smut, and in the F_2 generation of hybrids on Markton, 9 percent of the plants were infected. Approximately 50 percent of the F_3 progenies proved to be resistant, while the others contained infected individuals, a few being classified as susceptible.

Schattenberg (*9*) studied hybrids between Markton and three other varieties. He used two distinct strains of loose smut, one of which produced a comparatively weak infection of the susceptible parental varieties and the other a more severe infection. He explains his data for the F_3 progenies inoculated with the less virulent strain of smut on the basis of a three-factor difference, and the results obtained agree fairly well with expectations. Nevertheless, there was a shortage of susceptible progenies, with a corresponding slight excess of resistant ones. No clearly defined behavior was obtained with the other strain of smut.

The resistance to covered smut in hybrids in which Markton is one of the parents has been studied by Coffman et al. (*2*). The F_3 progenies of hybrids between Early Champion, Ligowa, Swedish Select, and Markton showed approximately one free from smut to three that contained infected individuals. Very few of these, however, contained highly susceptible families. The data for hybrids 50, 51, 52, and 53, as recorded herein, correspond rather closely with these results, whereas, in the hybrids of Scottish Chief, Iogold, Aurora, and Victory on Markton, about half the progenies were resistant while the other half contained infected individuals. The first three parental varieties of this second group showed comparatively low percentages of infection; Victory, however, proved very susceptible. Finally, in hybrids of Markton with Idamine and Silvermine, a larger proportion of resistant progenies was observed. In comparative experiments higher percentages of infection on an average were obtained from

Idamine than from Silvermine. These varieties are closely related and should have shown similar behavior.

Stanton, Reed, and Coffman (*11*) also recorded data on the inheritance of covered smut for certain hybrids involving Markton. No infected F_2 plants were observed in the Markton × Black Medsag hybrids, but in the F_3 generation infected individuals appeared in a few progenies. The crosses involving Iogold and Richland contained a few infected F_2 plants and also F_3 progenies with smutted individuals. An occasional F_2 plant of Cornellian × Markton was infected, and a few F_3 progenies contained smutted plants.

All investigators of crosses involving Markton have found a great preponderance of resistant F_3 progenies and a corresponding lack of susceptible ones. In a very few cases the results might be accounted for on the basis of a single-factor difference; in others at least two or three independent factors are involved.

The fact is mentioned earlier in this paper that in Markton, and especially in many of the F_3 plants from crosses on Markton, a blasted condition was observed, and apparently much of this blasting was due to the presence of the smut organism in the tissue of the plant. In view of this observed reaction, there perhaps might have been expected a much greater proportion of fully infected plants in the various hybrid generations. Evidently there is some factor or factors that prevent the full expression of susceptibility in hybrids involving Markton.

Results obtained from these hybrids on Markton afford an interesting contrast with those that have been recorded in crosses on Black Mesdag, a variety very resistant to the races of loose and covered smuts used in the present experiments. Reed (*7*) has recorded data for hybrids of Black Mesdag with a hull-less oat and with the Early Champion and Silvermine varieties, all three of which are very susceptible to both smuts. The data for the F_2 generations of the three hybrids indicated that resistance was dominant and that segregation for both smuts occurred on the basis of a 3 to 1 ratio. Furthermore, the data for the F_3 progenies harmonize quite well with those obtained for the F_2 generation. Again, there was a marked parallelism in inheritance of resistance to both smuts in the various F_3 progenies; a progeny resistant, segregating, or susceptible to one smut reacted similarly to the other smut. These results indicate that the same factor or closely linked factors were responsible for the resistance and susceptibility in these hybrids.

Stanton, Reed, and Coffman (*11*) reported results with hybrids between Black Mesdag and Monarch Selection (of Etheridge), the latter susceptible to the loose smut but resistant to the covered. The F_2 and F_3 data indicated segregation on the basis of a 3 to 1 ratio.

Reed (*8*) has studied crosses between Fulghum and Black Mesdag with reference to their reaction to the Fulghum race of loose smut. The results obtained indicated that resistance is dominant and that segregation occurred on the basis of a 3 to 1 ratio, the data for the F_2 and F_3 generations being in harmony.

Austin and Robertson (*1*) have recently reported a two-factor difference in the inheritance of resistance to covered smut in progenies of a Colorado No. 37 × Markton cross. Their F_2 data, however, indicate a three-factor relation. The data presented for the F_3

progenies also fit this interpretation, since 269 were fully resistant and 203 contained infected plants; therefore, a ratio of approximately 37 resistant to 27 segregating and susceptible is demonstrated. Since the publication of their results, Austin and Robertson [4] also have recognized the correctness of the three-factor interpretation.

SUMMARY

This paper presents the results obtained from five oat hybrid combinations involving crosses between Markton and varieties that differ in their behavior to the Missouri races of the loose and covered smuts. Markton is very resistant to both smuts and the crosses are arranged in three groups, depending upon the reaction of the other parential varieties to the smuts: (1) Varieties susceptible to both smuts, (2) a variety susceptible to the loose smut and resistant to the covered smut, and (3) a variety resistant to the loose smut and susceptible to the covered smut.

In group 1 there were four crosses, namely, Canadian × Markton, Early Champion × Markton and its reciprocal, and Victor × Markton. Separate series of F_2 plants of these crosses were inoculated with *Ustilago avenae*-Missouri and *U. levis*-Missouri, respectively. In the loose smut series the percentages of infection of the F_2 plants ranged from 8.6 to 16.0. Higher percentages of infection were obtained in the series inoculated with covered smut, these ranging from 20 to 32.5. Numerous F_3 progenies of each hybrid were inoculated with both loose and covered smuts. The results showed no agreement in the reaction of the progenies to the smuts; they reacted in a manner entirely independent of each other. The data for both F_2 and F_3 generations with loose smut may suggest a two-factor difference, while the corresponding data for the covered smut may suggest a single-factor difference. In both series, however, there was a noticeable lack of fully susceptible progenies.

Hybrid 56, Gothland × Markton, represents the second type of cross. The F_2 plants inoculated with loose smut gave 17.1 percent infection; in the F_3 generation, 72.6 percent of the F_3 progenies descended from uninoculated F_2 plants were segregating, the others resistant. No completely susceptible progenies were observed. Although both parents were resistant to covered smut, there were a few infected F_2 plants and some of the F_3 progenies contained smutted individuals.

Hybrid 60, Monarch × Markton, represents the third group. Although the parents were resistant to loose smut, a few F_2 plants were infected and smutted plants were found also among several F_3 progenies. In the case of the covered smut, 22 percent of the F_2 plants were infected and 74.4 percent of the F_3 progenies descended from uninoculated F_2 plants contained smutted individuals. These data may suggest a single-factor difference, but there is a noticeable lack of susceptible F_3 progenies.

[4] Letter from D. W. Robertson to T. R. Stanton, dated January 15, 1937.

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CHROMOSOMES OF MAIZE FROM NORTH AMERICAN INDIANS[1]

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INTRODUCTION

A knowledge of the morphological structure of the chromosomes of maize (*Zea mays* L.) and the closely related wild grasses should contribute to an understanding of the origin and distribution of this American cereal.

It was thought that a canvass of the strains of maize grown by the American Indians in isolated localities might disclose types of chromosomes intermediate between those of improved varieties and the wild ancestors of maize. Any additions to the known forms of maize chromosomes would be useful also in cytogenetic studies where there is now a need for visual markers to aid in the identification of parts of chromosomes as they pass from parent to offspring.

McClintock (*4*, *5*, *6*, *7*, *8*, *9*)[2] and others have described the morphological characters by which each chromosome of the set can be identified. The studies of these authors have been based largely on commercial maize or on derived genetic strains. These strains have been separated by many generations from the maize grown by the Indians, and it is possible, therefore, that the studies based on them do not give a complete picture of the morphological characters of maize chromosomes.

With the idea that the maize grown at the present time by the American Indians might have morphological features as yet undescribed, the author undertook to examine the chromosomes of such Indian strains as still are available. It is hoped, as opportunity offers, to extend this study, here limited to the Indian strains of the United States, to the maize of the Indians in Mexico, Central America, and South America.

MATERIAL AND METHODS

The source of material for this paper is a collection of maize from 33 North American Indian tribes of the United States. It should be understood that these tribes have to some extent intermingled as the result of their removal from their original lands to reservations.

Every effort has been made to obtain seed grown by Indians and free from admixture with commercial sorts, but, of course, in many instances there can be no certainty that such admixture has not taken place.

Carmine smears were used by the writer in making pollen mother cell preparations for study. It has been found that the best stained slides come either from fresh material or from material that has been

[1] Received for publication July 21, 1937; issued March 1938

[2] Reference is made by number (italic) to Literature Cited, p. 195.

killed in acetic-absolute alcohol (1:3) and held in absolute alcohol for about a day. Even under optimum conditions the chromosomes of some strains of maize stain with carmine less readily than do those of others. This difference between strains in the staining quality of chromosomes is more apparent in material stored for some time in absolute alcohol.

Identification of the individual chromosomes of the plants of the various strains of maize included in this study is based on their morphological characteristics, a method that may not be infallible. However, to test the chromosomes of each plant or even of each strain by crossing with plants in which each chromosome has a well-known marker would have been such a long process that the writer is presenting the following data without this additional check.

CHROMOSOME MORPHOLOGY

The detailed study of the individual maize chromosomes began with McClintock's attempt (*4*) to identify each chromosome from division phases of the microspore. Later McClintock (*6*) found that the early prophase of the first reduction division of the pollen mother cell was even more favorable for morphological studies. In this and later studies she has shown that every chromosome of the set is morphologically identifiable. These chromosomes have been given numbers from I to X beginning with the longest and ending with the shortest, and by combining cytological and genetic investigations, it has been possible to assign each linkage group in maize to its proper chromosome.

Maize chromosomes as seen in the midprophase of the first meiotic division of the pollen mother cells are in the paired thread condition. Stained with acetocarmine these 10 double threads are seen to differ in length, and each is divided into two arms by a fiber attachment region. In one chromosome these two arms are almost equal in length, but in others they are quite unequal. Knobs, when present at certain positions on these threads, are very useful in identifying the individual chromosomes. These major morphological characters of the maize chromosomes are often associated with other minor features that help to identify more readily and more definitely certain chromosomes.

The following presentation records the number and the position of knobs that aid materially in identifying each of the 10 chromosomes of maize or even fragments of them.

The data from 400 or more plants studied during the past three seasons are much too extensive to be given in detail, so they have been condensed and presented in table 1. In this table are listed the Indian tribes from which maize was obtained and their general location. Each of the 10 chromosomes is assigned a general section, and each section is subdivided into columns designating types 1, 2, 3, and 4. For each chromosome the number of plants without knobs is given in column 1, the number of plants with a knob on the short arm is given in column 2, the number of plants with a knob or knobs on the long arm is given in column 3, and the number of plants with a knob on the short arm and one or more knobs on the long arm is

given in column 4. Column 3 under the heading "Chromosome VIII" and column 4 under the heading "Chromosome VI" are further subdivided, since in the case of each of these chromosomes the long arm may be marked by more than one knob. The subdivision designated "a" gives the number of plants with the chromosome marked by a knob in the position nearest the end; "b", the number of plants with the chromosome marked by a knob in the second position from the end; and "c", the number of plants having the chromosome marked by a knob in the third position from the end. When knobs occur in more than one position on an arm, the number of plants is given under the combinations of ab, ac, bc, or abc, in accordance with the positions occupied.

The percentages at the foot of each of the columns under each chromosome of table 1 show the frequency of the chromosome types

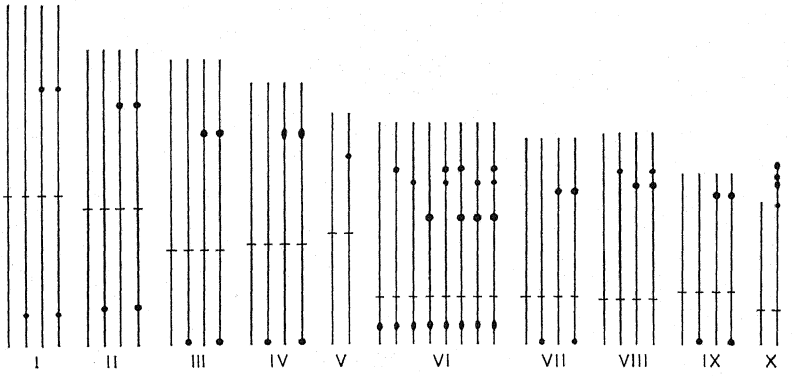


FIGURE 1.—Diagrammatic representation of the 10 chromosomes of maize showing types with or without knobs. Cross line indicates position of attachment of the spindle fiber.

described above, in the plants included in this study. These percentages may have little significance, however, since the number of plants of a strain was often very small.

The significance of the divisions and subdivisions under each chromosome of table 1 is made clearer when these divisions are examined in conjunction with figure 1. This figure presents diagrammatically the variations found in the position of the knobs of each of the 10 chromosomes. It shows also the relative length of each chromosome and the position of the attachment of the spindle fiber.

The size of any given knob on a chromosome, although constant for a particular strain, may vary when this knob is compared with that of an unrelated strain. Figure 1 shows all knobs about the same size, but actually the size may vary greatly.

Neither the data of table 1 nor the diagram of the chromosomes (fig. 1) differentiates between cases in which a knob is present on only one of the paired chromosomes and those in which both members of the pair are marked. This heterozygous condition is frequently difficult to determine and consequently in this general survey such cases are treated as though both chromosomes were marked by knobs at this position.

TABLE 1.—*Number of plants of the various chromosome types in maize strains grown by Indian tribes of different localities*

Indian tribe from which maize was obtained	Locality	Number of plants of indicated type [1] of chromosome—																																						B-type chromosome	
		I				II				III				IV				V		VI								VII				VIII				IX				X			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	3	2	4a	4b	4c	4ab	4ac	4bc	4abc	1	2	3	4	1	3a	3b	3ab	1	2	3	4	1	Abnormal	Without knobs	With knobs
Assiniboine	Montana	4	0	0	0	4	0	0	0	4	0	0	0	4	0	0	0	4	0	4	0	0	0	0	0	0	0	4	0	0	0	4	0	0	0	1	3	0	0	4	0	4	0
Crow	do	4	0	0	0	3	1	0	0	4	0	0	0	4	0	0	0	4	0	4	0	0	0	0	0	0	0	4	0	0	0	4	0	0	0	0	4	6	0	4	0	4	0
Sioux or Cheyenne	South Dakota	10	0	0	0	6	2	0	0	10	0	0	0	8	0	0	0	9	0	6	3	0	0	0	0	0	0	10	0	0	0	9	0	1	0	2	9	0	0	11	0	4	7
Mandan	North Dakota	11	0	0	0	11	0	0	0	11	0	0	0	11	0	0	0	6	5	3	8	0	0	0	0	0	0	11	0	0	0	11	0	0	0	3	8	0	0	11	0	11	0
Shoshone	Idaho	10	0	0	0	9	0	0	0	9	0	0	0	9	0	0	0	4	6	6	1	2	0	0	0	0	0	9	0	0	0	10	0	0	0	0	11	0	0	10	0	11	0
Menominee	Wisconsin	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	0	2	2	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	2	0	0	2	0	2	0
Sisseton	South Dakota	5	0	0	0	5	0	0	0	5	0	0	0	4	0	0	0	3	0	2	2	0	0	0	0	0	0	5	0	0	0	2	0	2	0	0	5	0	0	5	0	0	4
Sioux	North and South Dakota.	22	0	0	0	18	4	0	0	22	0	0	0	19	0	3	0	22	0	5	18	0	0	0	0	0	0	21	0	2	0	23	0	0	0	1	24	0	0	23	0	15	7
Ponca	Oklahoma	12	0	0	0	8	0	3	0	11	0	0	0	11	0	0	0	11	0	6	0	0	0	5	0	0	0	10	0	2	0	10	0	0	0	0	11	0	1	12	0	12	0
Potawatomi	Kansas	9	0	0	0	5	0	4	0	9	0	0	0	9	0	0	0	9	0	1	6	2	0	0	0	0	0	8	0	1	0	9	0	0	0	1	8	0	0	9	0	7	2
Sioux or Blackfeet	North Dakota	18	0	0	0	18	0	0	0	17	0	1	0	18	0	0	0	5	11	3	12	2	1	1	0	0	0	16	0	4	0	19	0	0	0	4	16	0	0	19	0	20	0
Chippewa	Minnesota and Wisconsin.	10	0	0	0	7	0	3	0	10	0	0	0	10	0	0	0	5	5	3	4	3	0	0	0	0	0	10	0	0	0	10	0	0	0	0	10	0	0	10	0	9	2
Cheyenne	Montana	2	0	0	0	2	0	0	0	2	0	0	0	0	2	0	0	2	0	1	1	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	2	0	0	2	0	2	0
Winnebago	Nebraska	12	0	0	0	2	8	2	0	12	0	0	0	12	0	0	0	12	0	4	8	0	0	0	0	0	0	12	0	0	0	11	0	0	0	0	13	0	0	12	0	4	9
Shawnee	Oklahoma	5	0	0	0	4	1	0	0	5	0	0	0	5	0	0	0	1	4	3	0	0	0	2	0	0	0	5	0	0	0	4	0	1	0	1	4	0	0	5	0	5	0
Sac and Fox	Iowa	14	0	0	0	8	3	4	0	14	0	0	0	15	0	0	0	7	7	5	4	0	0	3	0	0	2	10	0	5	0	14	0	0	0	2	11	0	2	15	0	10	5
Cheyenne or Arapahoe.	Oklahoma	11	2	0	0	6	0	8	0	13	0	0	0	5	0	8	0	13	0	5	9	0	0	0	0	0	0	7	0	7	0	6	9	7	0	5	8	0	0	13	0	12	3
Seminole	do	4	0	0	0	1	4	0	0	4	0	0	0	0	0	5	0	4	0	0	3	1	0	0	0	0	0	4	0	0	0	4	0	0	0	0	5	0	0	3	0	5	0
Five Civilized Tribes.	do	11	0	0	0	9	0	3	0	10	0	0	0	5	0	6	0	4	5	1	5	3	0	2	0	0	0	4	0	5	0	8	0	2	0	4	6	0	0	10	0	10	1
Osage	do	27	0	0	0	18	0	5	1	24	0	2	0	17	7	2	0	16	11	7	3	10	0	3	2	2	0	14	0	12	0	20	0	5	0	1	19	0	9	31	0	32	0
Cherokee	North Carolina	2	0	0	0	0	0	3	0	3	0	0	0	0	0	3	0	0	3	3	0	0	0	0	0	0	0	0	0	2	0	3	0	0	0	3	0	0	0	3	0	5	0
Pawnee	Oklahoma	8	0	2	0	2	4	2	2	10	0	0	2	8	0	2	0	8	3	6	4	0	0	0	0	0	0	7	0	3	0	8	0	2	0	0	9	0	3	11	0	11	2
Mission	California	2	2	0	0	0	0	4	0	2	0	0	0	2	0	0	0	3	0	0	2	2	0	0	0	0	0	2	0	0	0	1	2	0	0	0	4	0	0	3	0	5	0
Kiowa	Oklahoma	2	0	1	0	2	1	0	0	3	0	0	0	2	0	1	0	2	1	0	2	1	0	0	0	0	0	3	0	0	0	0	0	3	0	0	3	0	0	3	0	3	0
Zuni and Tewa	New Mexico	5	0	0	1	5	0	0	1	5	0	1	0	5	0	1	0	5	1	2	1	0	0	3	0	0	0	0	0	7	0	5	0	1	0	1	5	0	0	6	0	6	2
Cherokee	Oklahoma	5	2	0	0	1	3	0	4	6	0	0	0	3	0	4	0	5	2	3	4	0	0	0	0	0	0	3	0	5	0	1	0	6	0	8	1	0	0	9	0	9	0
Walapai	Arizona	12	0	0	0	1	0	10	0	5	0	7	0	5	0	5	0	5	3	5	6	0	0	0	0	0	0	4	0	8	0	7	0	5	0	4	5	0	2	13	0	14	0
Ute	Utah	3	3	0	0	5	0	2	0	2	1	3	3	5	0	4	0	2	6	1	6	0	1	2	0	0	0	6	0	2	0	3	0	1	5	2	6	0	2	3	3	9	2
Mescalero Apache	Arizona	8	3	7	3	4	1	10	8	7	0	8	10	5	1	11	2	5	19	8	7	2	0	2	4	1	1	11	0	12	0	7	0	15	1	6	18	0	0	17	6	21	9
Pima	do	14	2	0	0	0	0	3	7	5	0	11	0	0	0	14	0	0	16	0	1	0	0	16	0	0	0	0	3	7	5	0	0	8	8	0	17	0	0	17	0	17	0

| | | |

DESCRIPTION OF CHROMOSOMES

Any description of the chromosomes of maize is naturally built around the descriptions that have been published previously. Although the author appreciates that his colleagues are familiar with many of the different types of each chromosome described in this article, it may be helpful to others to call attention to previously published drawings and diagrams of maize chromosomes.

Chromosome I has been pictured diagrammatically without knobs by both Burnham (*1*) and McClintock (*8*), and by the latter author it has been shown with a small knob terminating the short arm. McClintock in three publications (*6, 7, 8*) has shown chromosome II with a knob on the long arm. The same author (*6, 8*) pictures diagram-

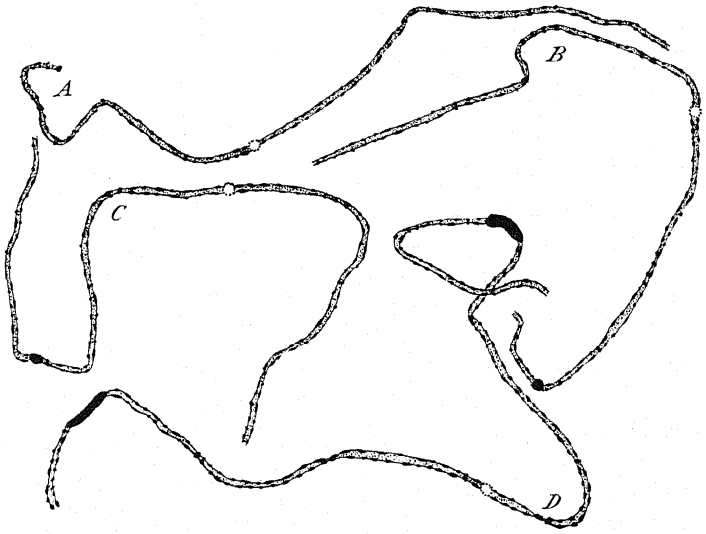


FIGURE 2.—Chromosome I: *A*, Type 1, without knob; *B*, type 2, with knob on short arm; *C*, type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

matically chromosome III without knobs. Chromosome IV is shown diagrammatically by McClintock (*8*) with a knob on the long arm in its characteristic position. McClintock (*8*) pictures chromosome V with a knob on the long arm and Rhoades (*11, 12*), in his cytogenetic research, has used both the knobless and the knobbed type of chromosome V.

Burnham (*1*) shows in his diagram of chromosome VI no knobs on its long arm, while McClintock (*6, 8, 9*) has pictured this chromosome with both one and two knobs on the long arm. Chromosome VII is shown in McClintock's diagrams (*6, 8*) with a knob on the long arm and with knobs on both arms. The same author (*5, 8*) pictures chromosome VIII with one knob and with two knobs, a larger and a smaller one, the latter nearer the end of its long arm. Chromosome IX has types with and without a knob terminating the short arm in the maize studied both by McClintock (*5, 8, 9*) and by Creighton and McClintock (*2*). The tenth or shortest chromosome has been shown diagrammatically without knobs by McClintock (*6, 8*).

CHROMOSOME I

The fact that chromosome I is the longest of the maize chromosomes serves to distinguish it from the other chromosomes. From figures 1 and 2 it is apparent that chromosome I is not always a long chromosome marked only by chromomeres and the spindle fiber attachment but may have in addition a knob or dark staining enlargement near the end of the short arm and another at a position approximately one-fourth the length of the chromosome from the end of the long arm. When this chromosome has a knob or knobs at one or both of these positions, the author has found it rather easy to identify. Frequently this chromosome has the terminal chromomere of the short arm enlarged

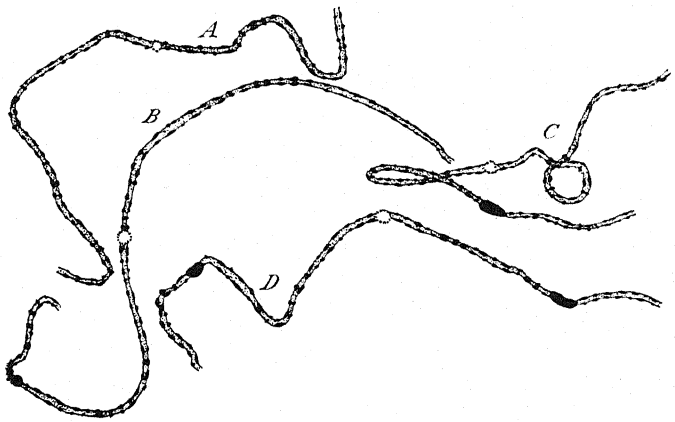


FIGURE 3.—Chromosome II: *A*, Type 1, without knob: *B*, type 2, with knob on short arm; *C*, type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

sufficiently to be a distinguishing feature, but since the enlargement of this chromomere never seems to be prominent it has not been treated as a knob.

CHROMOSOME II

Four types of chromosome II are shown in figures 1 and 3. This chromosome is most readily identified when it has a knob on both arms. The unmarked type and the type with only a knob on the long arm may be confused with the two types of chromosome V. This confusion disappears when the two chromosomes are carefully scrutinized. Chromosome II has arms more unequal in length, and when the long arm is marked by a knob the knob is a little farther from the end of the chromosome than is the case with chromosome V.

CHROMOSOME III

Of the long chromosomes, chromosome III is most readily identified because the long arm is approximately double the length of the short arm. The four types of this chromosome are shown in figures 1 and 4. When a knob is present on the long arm it is approximately one-fourth of the length of the chromosome from the end. The knob on the short arm has been pictured as terminal, but in those cases in which this knob was reduced in size to a prominent chromomere it was the third chromomere from the end that was enlarged.

CHROMOSOME IV

Chromosome IV has few morphological characteristics to distinguish it sharply from several of the other chromosomes, but in spite of this fact it has never seemed difficult to identify. Figures 1 and 5 show the four types of this chromosome. In those plants in which the short arm showed a knob, this knob was so similar to the knob on the

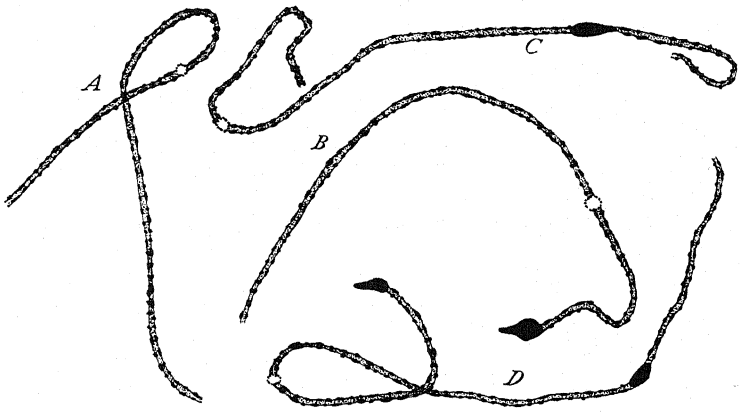


FIGURE 4.—Chromosome III: *A*, Type 1, without knob; *B*, type 2, with knob terminating short arm; *C* type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

short arm of chromosome III, both in position and in size variations, that for a time the author was somewhat confused, but uncertainty of identification was dispelled when both chromosomes III and IV, with knobs on the short arm, were found in the same plant and were distinguishable not only by the difference in position of the spindle

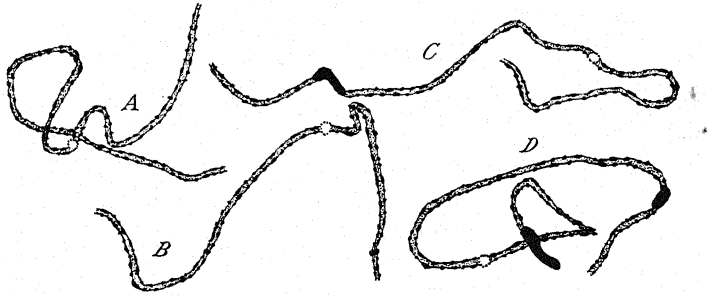


FIGURE 5.—Chromosome IV: *A*, Type 1, without knob; *B*, type 2, with small knob near end of short arm; *C*, type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

fiber attachment but also by the presence of knobs on the long arms. The knob on this arm of chromosome III is farther from the end than is that on chromosome IV.

CHROMOSOME V

The two arms of chromosome V are nearly equal in length, and it is this characteristic that serves best as an identifying feature. The likelihood of confusing this chromosome with chromosome II has been discussed above. The absence of a knob on the short arm of

chromosome V in any of the Indian strains included in this study leaves only two types of this chromosome to be illustrated in figures 1 and 6.

CHROMOSOME VI

The so-called satellite or sixth chromosome is readily identified because of its attachment at prophase to the nucleolus. Figure 7

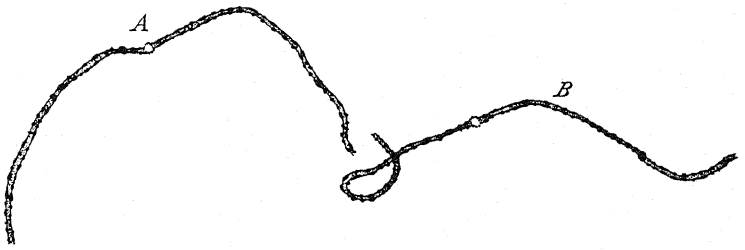


FIGURE 6.—Chromosome V: *A*, Type 1, without knob; *B*, type 3, with knob on long arm. × 1,500.

shows the eight types that the author has observed. The long arm of this chromosome is only rarely seen without one or both of the small knobs nearest the distal end, while the knob at about the mid-

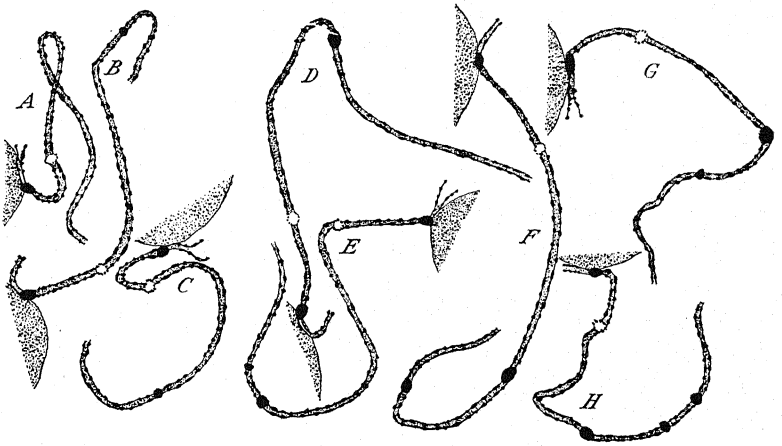


FIGURE 7.—Chromosome VI: *A*, Type 2, without knob on long arm; *B*, type 4a, with knob on long arm in position nearest the end; *C*, type 4b, with knob on long arm in second position from the end; *D*, type 4c, with knob on long arm in third position from the end; *E*, type 4ab, with knobs on long arm in first and second positions; *F*, type 4ac, with knobs on long arm in first and third positions; *G*, type 4bc, with knobs on long arm in second and third positions; and *H*, type 4abc, with three knobs on long arm. × 1,500.

point of this arm, although usually prominent, is less frequently present.

CHROMOSOME VII

McClintock described to the author a universal characteristic of chromosome VII that makes its identification rather simple. On the long arm adjacent to the spindle fiber attachment the chromosome is broad and stains deeply with carmine. Of the four types shown in figures 1 and 8 the types with a knob terminating the short arm are

rarely present and were found in only one of the strains given in table 1.

CHROMOSOME VIII

Chromosome VIII is distinguished most readily from the other chromosomes when it is marked by two knobs on the long arm. These knobs are usually unequal in size and the knob nearer the end is never

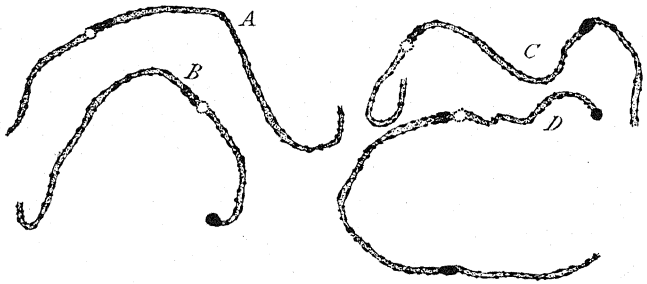


FIGURE 8.—Chromosome VII: *A*, Type 1, without knobs; *B*, type 2, with knob terminating short arm; *C*, type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

very prominent. Some types of chromosome VIII that are likely to be confused with chromosome VII are distinguishable from them by the absence of the heavily stained region adjacent to the spindle

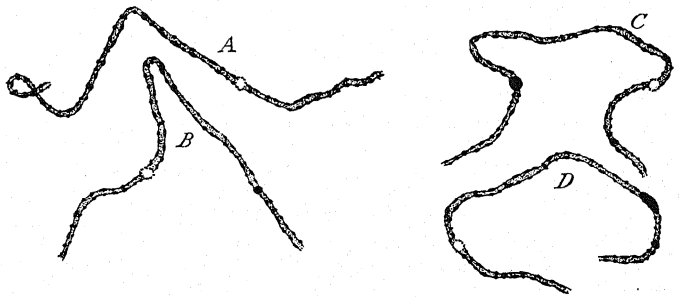


FIGURE 9.—Chromosome VIII: *A*, Type 1, without knob; *B*, type 3a, with small knob at position nearest the end of long arm; *C*, type 3b, with knob at position second from the end of long arm; and *D*, type 3ab, with two knobs on long arm. × 1,500.

fiber attachment. Figure 9 shows the four types that occur in the strains given in table 1.

CHROMOSOME IX

Cytogeneticists have found chromosome IX very useful because it is so readily identified and because its linkage group is one of the best known. This chromosome "stands out" from the shorter just as chromosome III "stands out" from the longer chromosomes because the short arm is approximately one-half the length of the long arm. The knob terminating the short arm, though occurring in several sizes, is so frequently present that it almost leaves the impression that chromosome IX is incomplete without it. Figure 10 shows three types of chromosome IX; the type with a knob near the end of the long arm was found in only a few strains.

CHROMOSOME X

Chromosome X, the shortest of all maize chromosomes, is shown in figure 11, *A*. The author in a few instances has found on this chromosome a prominent chromomere near the end of the long arm, but in the present discussion this enlargement has not been treated as a knob. In figure 11, *B*, is shown an abnormal type of chromosome X, a type similar to that already described by the author (*3*) as occurring in both teosinte and maize. This abnormal type of the tenth chromosome has an additional piece on the end of the long arm, approximately

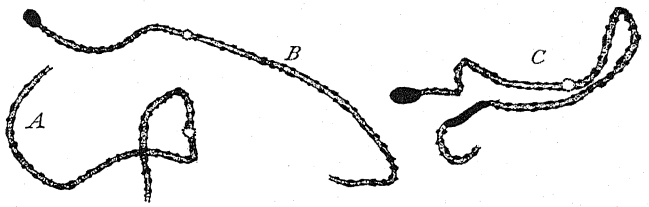


FIGURE 10.—Chromosome IX: *A*, Type 1, without knob; *F*, type 2, with knob terminating short arm; and *C*, type 4, with knobs on both arms. × 1,500.

the length of the short arm. In some plants this abnormal piece may be marked by a large knob near the end as shown in figure 11, *B;* in other plants this region may show two adjacent knobs instead of the one large knob, and in still other plants there also may be a knob terminating the chromosome (fig. 1). In these abnormal types of chromosome X there are usually one or two knoblike enlargements near the end of the normal part of the chromosome. Additional study of the tenth chromosome may show that these positions on the



FIGURE 11.—Chromosome X: *A*, Normal type; *B*, one of the abnormal types. × 1,500.

normal tenth chromosome have a tendency to form knobs. Nothing in the character of the additional piece found attached to the tenth chromosome has suggested a solution of its origin.

POSITION AND NUMBER OF KNOBS

If in a single plant all positions on the chromosomes that have been observed to have knobs were so occupied, that plant would have 18 knobs. This disregards the knoblike enlargement at the nucleolar attachment region of chromosome VI and considers only the knobless type of chromosome X. Such a plant would have for its chromosome complement the nine right-hand types of the nine longest chromosomes shown in figure 1 and the normal knobless type of the tenth chromosome.

Other knob-forming positions may be found, but the present discussion is limited to the 18 observed positions. In addition to being restricted to definite positions, knobs in some instances are also

characterized by their size and shape, although in other instances the size of a knob differs in different maize strains.

Considering, therefore, these 18 knob positions, it has been found that some plants are without knobs at any of the positions and that other plants may have as many as 14 of the positions marked by knobs. It should be comparatively easy, by means of appropriate combinations, to produce plants with knobs at all 18 positions or to maintain strains without knobs.

To visualize better the relationship between these 18 positions and the data presented in table 1, these data have been tabulated differently in table 2. The values in the 18 columns, under the 9 chromosomes that are marked by knobs, indicate the frequency that each position has been marked by a knob. A frequency of 0 percent indicates that no knob has been found at this position among all the plants of the strain; a frequency of 100 percent indicates that a knob has been found at this position in all plants of the strain.

If a strain of maize were uniform all 18 positions would show a frequency for each position of either 0 or 100 and never an intermediate value. Such uniformity may be reached by prolonged pure seeding or selfing. The author's material was not taken from pure lines and frequently the data for a strain combine the data from several substrains that, although coming from the same Indian tribe, may have been received at different times and from different localities. Consequently similar and not identical morphological markers on the chromosomes were to be expected in any particular maize strain.

TABLE 2.—*Percentage of plants with knobs at each of the 18 positions on 9 of the 10 chromosomes of maize from 33 Indian tribes*

Indian tribe from which maize was obtained	Percentage of plants having specified chromosome with knobs at indicated position								
	I		II		III		IV		V
	Short arm	Long arm	Short arm	Long arm	Short arm	Long arm	Short arm	Long arm	Long arm
Assiniboine	0	0	0	0	0	0	0	0	0
Crow	0	0	25	0	0	0	0	0	0
Sioux or Cheyenne	0	0	25	0	0	0	0	0	0
Mandan	0	0	0	0	0	0	0	0	45
Shoshone	0	0	0	0	0	0	0	0	60
Menominee	0	0	0	0	0	0	0	0	100
Sisseton	0	0	0	0	0	0	0	0	0
Sioux	0	0	18	0	0	0	0	14	0
Ponca	0	0	0	27	0	0	0	0	0
Potawatomi	0	0	0	44	0	0	0	0	0
Sioux or Blackfeet	0	0	0	0	0	6	0	0	69
Chippewa	0	0	0	30	0	0	0	0	50
Cheyenne	0	0	0	0	0	0	100	0	0
Winnebago	0	0	67	17	0	0	0	0	0
Shawnee	0	0	20	0	0	0	0	0	80
Sac and Fox	0	0	20	27	0	0	0	0	50
Cheyenne or Arapahoe	15	0	0	57	0	0	0	62	0
Seminole	0	0	80	0	0	0	0	100	0
Five Civilized Tribes	0	0	0	25	0	0	0	54	56
Osage	0	0	4	25	0	8	27	8	41
Cherokee (North Carolina)	0	0	0	100	0	0	0	100	100
Pawnee	0	20	60	40	17	17	0	20	27
Mission	50	0	0	100	0	0	0	0	0
Kiowa	0	33	33	0	0	0	0	33	33
Zuni and Tewa	17	17	17	17	0	17	0	17	17
Cherokee (Oklahoma)	28	0	88	50	0	0	0	57	28
Walapai	0	0	0	91	0	58	0	50	38
Ute	50	0	0	28	44	67	0	44	75
Mescalero Apache	28	48	39	78	40	72	16	68	79
Pima	12	0	70	100	0	69	0	100	100
Hopi	100	43	80	40	0	100	0	100	100
Pueblo	100	100	100	75	0	100	0	80	100
Navajo	79	54	90	92	26	95	0	98	96

TABLE 2.—*Percentage of plants with knobs at each of the 18 positions on 9 of the 10 chromosomes of maize from 33 Indian tribes*—Continued

Indian tribe from which maize was obtained	Percentage of plants having specified chromosome with knobs at indicated position									Average number of knobs per plant
	VI			VII		VIII		IX		
	Long arm			Short arm	Long arm	Long arm		Short arm	Long arm	
	a [1]	b [2]	c [3]			a [1]	b [2]			
Assiniboine	0	0	0	0	0	0	0	75	0	0.75
Crow	0	0	0	0	0	0	0	100	0	1.25
Sioux or Cheyenne	33	0	0	0	0	0	10	82	0	1.50
Mandan	73	0	0	0	0	0	0	73	0	1.91
Shoshone	11	22	0	0	0	0	0	100	0	1.93
Menominee	0	0	0	0	0	0	0	100	0	2.00
Sisseton	50	0	0	0	0	0	50	100	0	2.00
Sioux	78	0	0	0	10	0	0	96	0	2.16
Ponca	45	45	0	0	17	0	0	100	8	2.42
Potawatomi	67	22	0	0	11	0	0	89	0	2.33
Sioux or Blackfeet	68	16	5	0	20	0	0	80	0	2.64
Chippewa	40	30	0	0	0	0	0	100	0	2.50
Cheyenne	50	0	0	0	0	0	0	100	0	2.50
Winnebago	67	0	0	0	0	0	0	100	0	2.51
Shawnee	40	40	0	0	0	0	20	80	0	2.80
Sac and Fox	64	36	14	0	33	0	0	87	13	3.44
Cheyenne or Arapahoe	64	0	0	0	50	0	54	62	0	3.64
Seminole	75	25	0	0	0	0	0	100	0	3.80
Five Civilized Tribes	64	45	0	0	56	0	20	60	0	3.80
Osage	30	56	15	0	46	0	20	97	31	4.08
Cherokee (North Carolina)	0	0	0	0	100	0	0	0	0	4.00
Pawnee	40	0	0	0	30	0	20	100	25	4.16
Mission	50	50	0	0	0	66	0	100	0	4.16
Kiowa	67	33	0	0	0	0	100	100	0	4.32
Zuni and Tewa	67	50	0	0	100	0	17	83	0	4.36
Cherokee (Oklahoma)	57	0	0	0	62	0	86	11	0	4.67
Walapai	54	0	0	0	67	0	42	64	18	4.82
Ute	80	20	10	0	75	56	67	80	20	7.16
Mescalero Apache	56	24	24	0	52	4	70	75	0	7.73
Pima	100	94	0	53	80	50	100	100	0	10.28
Hopi	86	43	100	0	80	0	100	78	0	10.50
Pueblo	100	33	33	0	100	0	100	100	33	11.54
Navajo	81	74	40	0	98	42	98	91	37	11.91

[1] Knob position nearest the end.
[2] Knob position second from the end.
[3] Knob position third from the end.

The average number of positions marked by knobs in a given strain is shown in the last column of table 2. Several methods were tried to derive a satisfactory average for the number of knobs per chromosome per plant and per strain. If the author had been able to make a drawing of every chromosome of all plants the total number of knobs on the 10 chromosomes of each plant of a strain could have been averaged, but to drop out all plants of a strain that did not have a drawing of every chromosome was unsatisfactory because in so many instances those plants with a drawing of all 10 chromosomes were the plants with the fewer knobs on the chromosomes. Consequently an attempt was made to devise a method that would combine the complete data from some plants with the fragmentary data from others.

A fairer method that would use data from all plants whether complete or fragmentary seemed to be to consider each knob position separately, as in table 2. This method takes into account each knob position on each chromosome, and when a particular knob position on a chromosome is marked by a knob in all plants of a strain the knob frequency is 100 percent for this position, while if this

position is never marked by a knob the frequency is 0. In many instances part of the plants of a strain have a knob at a certain position and part are knobless, thus giving a knob frequency intermediate between 0 and 100 percent. The sum of the knob frequency of all 18 positions thus determined independently is taken as the average frequency of knobs for a strain.

Averaging the number of knobs on the chromosomes of a strain should be restricted, of course, to those strains in which the number of knobs on the chromosomes of the individual plants have a random distribution about their mean. The number of plants studied from a strain was frequently too meager to test such a distribution. In a few strains, however, it was evident that certain substrains should not be combined. The data from such strains that have been averaged in table 2 have also been subdivided and presented in table 3.

TABLE 3.—*Percentage of plants with knobs at each of the 18 positions on 9 of the 10 chromosomes of maize from four Indian strains*

Indian tribe from which maize was obtained	Percentage of plants having specified chromosome with knobs at indicated position								
	I		II		III		IV		V
	Short arm	Long arm	Short arm	Long arm	Short arm	Long arm	Short arm	Long arm	Long arm
Ute	0	0	0	0	0	0	0	0	0
Pawnee	0	0	50	25	0	0	0	0	0
Mescalero Apache	0	0	0	0	0	0	0	0	0
Zuni and Tewa	0	0	0	0	0	0	0	0	0
Mescalero Apache	0	38	12	75	20	50	0	83	70
Ute	75	0	0	40	57	86	0	57	100
Mescalero Apache	54	64	62	92	62	100	27	73	100
Pawnee	0	100	100	100	100	100	0	100	100
Tewa and Zuni	100	100	100	100	0	100	0	100	100

Indian tribe from which maize was obtained	Percentage of plants having specified chromosome with knobs at indicated position								Average number of knobs per plant
	VI			VII		VIII		IX		
	Long arm			Short arm	Long arm	Long arm		Short arm	Long arm	
	a[1]	b[2]	c[3]			a[1]	b[2]			
Ute	100	0	0	0	0	0	0	0	0	1.00
Pawnee	25	0	0	0	0	0	0	100	0	2.00
Mescalero Apache	0	100	0	0	0	0	0	100	0	2.00
Zuni and Tewa	60	40	0	0	100	0	0	80	0	2.80
Mescalero Apache	50	0	0	0	0	0	70	33	0	5.01
Ute	75	25	12	0	33	71	86	100	25	8.42
Mescalero Apache	69	31	46	0	100	9	82	100	0	10.71
Pawnee	100	0	0	0	100	0	100	100	100	12.00
Tewa and Zuni	100	100	0	0	100	0	100	100	0	12.00

[1] a=Knob position nearest the end.
[2] b=Knob position second from the end.
[3] c=Knob position third from the end.

By the foregoing method the author has attempted to arrive at a figure from the data available that may be used as a measure of the knobbiness of the chromosomes in each of the 33 strains of Indian corn. The values in the last column of table 2 showing the average

number of knobs for each of the 33 strains are also presented graphically in figure 12. The range is from less than 1 to almost 12 knobs.

Maize from the Pima, Hopi, Pueblo, and Navajo Indians is shown in table 2 as having an average number of knobs per plant greater than 10. This group coming from adjoining regions suggests a source of material should prominent knob markers on most of the chromosomes be desired.

The same tendency toward knobs is found in most of the plants from the Mescalero Apache and in some plants from the Zuni and

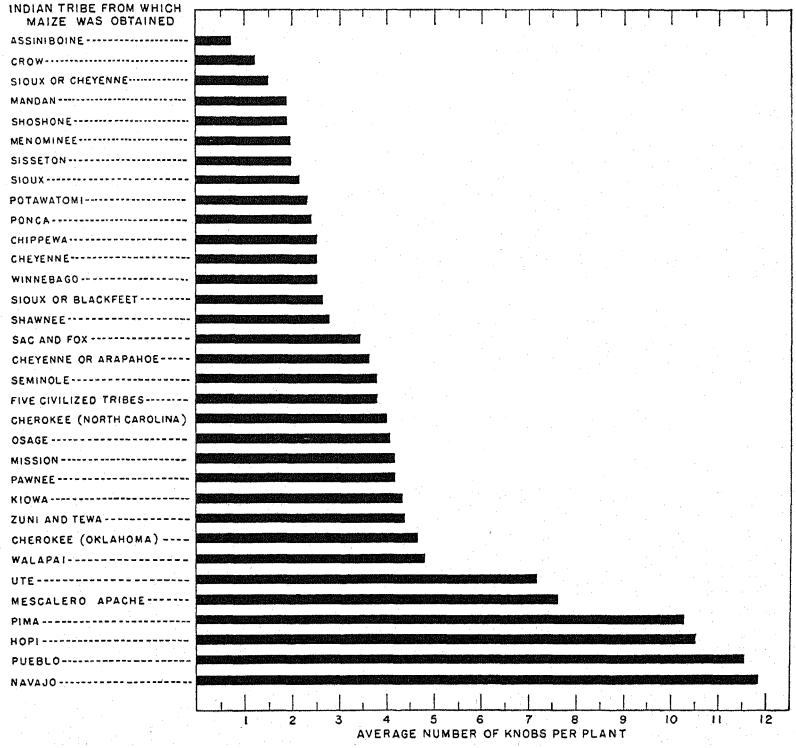


FIGURE 12.—Average number of knobs per plant on the chromosomes of 33 strains of Indian corn.

Tewa,[3] Ute, and Pawnee Indians. These strains are the four that have been subdivided in table 3 because the plants with many knobs were clearly separated from those with few knobs.

In marked contrast to the prevalence of knobs on the chromosomes of maize from these southwestern localities is the small number of knobs on the maize from the Indians along the northern border of the United States. Of the first 16 tribes listed in table 2, 14 are northern tribes. All of these except the Shoshone belong either to the Siouan or to the Algonquin families. The 14 strains of maize from these northern tribes have very few knobs on the chromosomes, and the whole range is only from 0.75 to 3.44 knobs per plant.

Nine strains of maize given in table 1 are from Indians of Oklahoma, the melting pot of the northern and eastern Indian tribes. Table 2

[3] Seed from this strain was collected from several reservations and then grown in Colorado.

shows that the number of knobs on the chromosomes of these nine Oklahoma strains ranges from 2.42 to 4.67 per plant. These strains, although intermediate in geographical position between those of the northern and those of the southwestern Indian tribes described above, are not intermediate when the number of knobs on the chromosomes of the plants is compared.

Only one strain of maize from the Southeast is shown in table 2. The average number of knobs on the chromosomes of maize of the Cherokee Indians from North Carolina is four per plant. It is scarcely justifiable to use the data from this one strain as indicative of the morphological characteristics of the chromosomes of the Indian corn of the entire southeastern area. This one example, when combined with data from the southeastern tribes now living in Oklahoma, seems, however, to indicate that the maize from the Southeastern States tends to have more knobs on its chromosomes than does the maize of the Indian tribes of the North. In general the maize of the Oklahoma Indians seems to be a blend of that from the Indians who lived north, east, and southeast of this central region.

Only in the maize from the Pawnee Indians of Oklahoma is there indication of contamination with that of the Indian tribes to the Southwest. A few plants in the author's collection coming from the Pawnee Indians were found to have the chromosomes generally marked by knobs. These knobs are so similar in number and size to the knobs on maize strains of the Southwest and the general morphological features of the chromosomes of these few plants are so like the chromosomes of the maize of adjacent New Mexican Indians that a relationship is suggested.

Mixing similar to that for the maize of the Oklahoma Indians seems to have begun in the maize of the Ute, Zuni and Tewa, and Mescalero Apache Indians, but in these latter tribes it is the maize of the North and of the Southwest that have recently been brought together in the same tribes. Only in the maize from the Mescalero Apache is there indication that the mixing has been followed by hybridization giving strains with the number of knobs per plant intermediate between northern and southwestern strains. The view that there has been a mixing of the maize from the northern with that from the southwestern Indians is supported in one instance by anthropologists who consider the Zuni and Tewa Indians to be a mixture of Pueblo and some tribe of northern Indians.

The seed from the Walapai Indians came from the reservation on the west border of Arizona and that from the Mission Indians from the Pala Reservation in southwest California. The strains from both tribes have relatively few knobs on their chromosomes and hence differ widely from the varieties grown by their adjacent neighbors on the east.

Insofar as the number of knobs per plant on the chromosomes of maize from various Indian tribes can be considered as indicative of relationship of the maize grown by these Indians, the author would group the various strains into (1) the southwestern group with knobs prevalent, (2) the northern group with knobs rare, and (3) the southeastern group with relatively few knobs, although more than were found on the chromosomes of the northern group.

The author's interpretation of the data supplied by the maize of the Walapai and Mission Indians of the Southwest is that they ha-

chromosomes with morphological characters more like the maize of the neighboring tribes to the north than like that of tribes to the east.

To visualize more clearly, from a geographical standpoint, the data just discussed, the graph of figure 13 has been prepared. In this the number of knobs per plant for the maize of each State has been averaged, though occasionally two adjacent States are considered together. This graph shows clearly the prevalence of knobs on the chromosomes

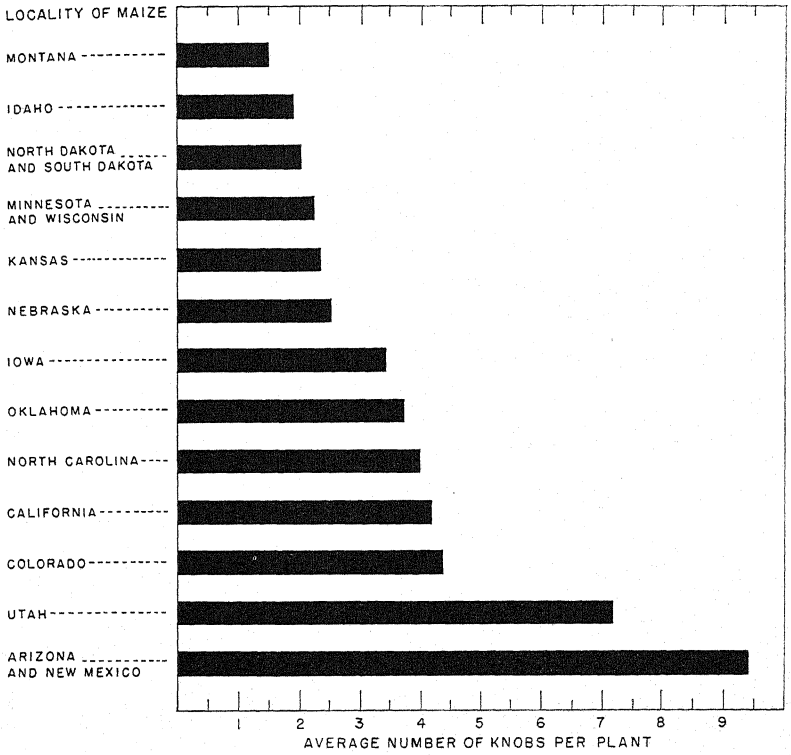


FIGURE 13.—Average number of knobs per plant on the chromosomes of maize from 16 States represented by 13 bars.

in the maize strains from Arizona and New Mexico, and the small number of knobs from the Northern States.

The maize strains of the eastern Indian tribes and those of the tribes from the North have been so jumbled with each other in their Oklahoma location that they show only in a general way a slightly greater prevalence of knobs on their chromosomes than the more isolated northern maize. The author feels, however, after a preliminary study of maize from the West Indies, that there is a resemblance between the chromosomes of the maize from these islands and those of the maize grown by the southeastern Indians. The possibility is thus suggested that maize may have been introduced into the United States through two sources—Mexico and Florida or the Carolinas.

This general study of the knobs on the chromosomes of strains grown by the Indians, combined with a previous study (*3*) of the

chromosomes of the nearer maize relatives, suggests to the author that knobs are a characteristic feature of the chromosomes of maize and its relatives growing in their native habitats. Strains of maize found farthest from Central America and Mexico have lost many of the knobs that mark so prominently the chromosomes of maize and its relatives growing in those regions.

ABNORMALITIES

Those maize strains in which the number of knobs on the chromosomes is highest, have, as one would expect, knobs at positions not previously recorded. It was also in these strains having many knobs that the author found abnormal types of chromosome X. Abnormal types were first seen in an annual teosinte from Mexico and have already been described by the author (*3*). Twenty-three plants with the long form of the tenth chromosome were found in the maize strains from the Navajo, Pueblo, Mescalero Apache, and Ute Indians. In one substrain of maize from the Navajo Indians the tenth chromosome was normal in length but had a prominent chromomere near the end of the long arm at a position usually marked by an enlargement in the longer abnormal form of this chromosome.

A study of the association between the number of knobs on the chromosomes and the occurrence of B-type chromosomes was made. Dividing the data into two groups, one containing those plants with more than 7 knobs and the other those plants with less than 7 knobs, showed that in the 145 plants with more than 7 knobs only 9, or 6.2 percent, carried B-type chromosomes, whereas in the 286 plants with less than 7 knobs 57, or 20 percent, carried B-type chromosomes. This greater prevalence of B-type chromosomes in plants with few knobs was illustrated further when after the subdivision and examination of the maize strains from the Mescalero Apache, the Zuni and Tewa, Ute, and Pawnee tribes, it was found that in every case the plants with B-type chromosomes were plants with few knobs on their chromosomes.

With one exception B-type chromosomes in all plants appeared identical and were marked by darkly stained areas similar to those pictured by McClintock (*6*). In the Pawnee strain, however, diminutive B-type chromosomes, similar to those described by Randolph (*10*), were found. These diminutive chromosomes were frequently associated with the normal B-type chromosomes, clearly proving their relationship. When, however, they were found unassociated with the normal B-type chromosomes it could be seen that each was a spindle fiber attachment and a short thread three or four chromomeres long.

SUMMARY

The presence of knobs on the threadlike paired chromosomes at prophase of the first reduction division of the pollen mother cells of maize is one of the most useful morphological characters in identifying the chromosomes.

A survey of the maize strains from 33 Indian tribes was successful in adding a few more prominent knobs to those already known and in the discovery of a form of the tenth chromosome previously unknown in maize.

The data show very few knobs on the chromosomes from the maize of the northern Indian tribes, a slightly higher number of knobs on the chromosomes of maize from the Indian tribes of the Southeast, and many knobs on the chromosomes of practically all the maize from Arizona and New Mexico.

The number of knobs on the chromosomes of the maize of the Indians of the United States increases as the tribes approach Mexico, where the much-knobbed chromosomes of maize and maize relatives occur.

The number of knobs on the chromosomes of a strain of Indian corn may give a clue to the geographical origin of the strain.

B-type chromosomes, although prevalent in most strains studied, were found more frequently in plants with less than seven knobs than in plants with more than seven knobs on their chromosomes.

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ESTABLISHMENT AND SPREAD OF MOLDS AND BACTERIA ON COTTON ROOTS BY SEED AND SEEDLING INOCULATION[1]

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INTRODUCTION

Investigations conducted in the Blackland prairie section of Texas by Jordan, Dawson, Skinner, and Hunter (*5*),[2] of the Division of Soil Fertility Investigations, Bureau of Plant Industry, indicated a need for a study of the changes in the microflora attending certain crop practices. These and subsequent projects, both field and laboratory, carried out in cooperation with the Division of Soil Microbiology, Bureau of Plant Industry, and the Department of Botany and Bacteriology of the University of Texas, have indicated the desirability of investigating the relation of native and other organisms to the soil-borne pathogen *Phymatotrichum omnivorum* (Shear) Duggar.

The general relations of the pathogen to the cotton plant are well described in the literature. The results of various investigations of biological control of soil pathogens have been summarized by Waksman (*9*). The first step in the practical application of biological control is the establishment of desirable organisms in the soil under field conditions. That soil inoculation has been successful with the rhizobia (*1*) is well known, and establishment is inferred in the work reported where biological control of soil pathogens has been accomplished.

This paper briefly reports the results obtained in the analysis of plant roots and adhering soil for introduced bacteria and molds following the inoculation of cotton seed and seedlings. It appeared desirable to conduct the experiment in a field infested with root rot so that both infected and healthy plants would be available for comparison. The field plan was not sufficiently comprehensive to permit interpretation with assurance of possible indications of biological control due to the introduced organisms. The immediate objective of the work was to determine whether or not the bacteria and molds inoculated upon seed and seedlings are in this way established in the soil.

PROCEDURE AND MATERIAL

FIELD PLOTS

The experimental field was divided into three sections, in each of which inoculation was accomplished by a different method, designated as treatment 1, 2, or 3. Treatment 1 included eighteen 300-foot rows; the other treatments, eighteen 100-foot rows each.

[1] Received for publication August 20, 1937; issued March 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 207.

TEST ORGANISMS

Three molds and two bacteria antagonistic to *Phymatotrichum omnivorum* in laboratory culture were selected.

Aspergillus luchuensis.—This organism was isolated from Texas soil, where heavy infestation had prevailed for years but from which root rot had been retreating for several seasons. It was selected in preference to other black aspergilli because its purple-brown color and single row of sterigmata facilitate identification.

Pseudomonas fluorescens.[3]—This strain was isolated from Texas soil about live, healthy roots of cotton in soil that had been continuously free from root rot.

Penicillium luteum.[3]—This fungus was isolated from roots of green plants taken from an area which, insofar as records reveal, had never been infested with root rot. Culture characteristics make fairly certain the identification of the fungus when reisolated. Results of Wehmer (*10*) and others (*6*) further justify its inclusion.

Achromobacter radiobacter.[3]—The culture used was obtained from the University of Wisconsin. Because of its association with rhizobia of legume roots, this form has long held the interest of soil microbiologists.

Trichoderma lignorum.[3]—The culture used was obtained from the same area as the *Aspergillus*. In view of the interesting observations of Weindling (*11, 12, 13*) and others on its parasitism of soil-borne pathogens, this fungus was selected.

INOCULATION AND PLANTING

Treatment 1.—Mebane cotton seeds were soaked for 1 hour in 10-day cultures of the respective organisms in nutrient broth. (The molds were cultured in glucose-nutrient broth.) Growth masses and pellicles were well broken by shaking them with glass beads. When the seeds were taken from the culture, as much of the liquid as possible was removed by squeezing lightly, and the moist masses of seeds were spread over paper and partly dried. Treated in this way the seeds were dry enough to pass through the planter, but moist enough to carry the inoculum. Two 300-foot rows for each of the five organisms, alternating with two control rows of uninoculated seed (treated with sterile broth), were planted.[4]

Treatments 2 and 3.—Untreated seeds were planted for subsequent seedling inoculation.[4] In treatment 2,[4] seedlings were inoculated with heavy broth cultures of the respective organisms, sterile broth being added to the control rows. The roots were exposed by a hand trowel and the culture (25–50 cc) poured about the root crown, after which the soil was carefully replaced. For treatment 3,[5] 10-day cultures inoculated on potato-dextrose agar at pH 6.5 (2.5-cm cubes) were introduced as in treatment 2, sterile agar being placed in the control rows.

LABORATORY PROCEDURE

Samples selected for analysis[6] were lateral roots and taproots from both single plants and groups of plants and, in some cases, soil adjacent

[3] This species name designates a great group and is used here in the sense of an aggregate of strains.
[4] Seed planted April 17, 1936.
[5] Treatment introduced June 15, 1936.
[6] Collection of samples, July 9 to August 8, 1936.

to the roots. The root or soil was carefully exposed and removed to sterile soil cans. The samples were usually analyzed within 24 hours after collection; during any delay, however, they were refrigerated at 6.0° C.

Each sample was weighed and then washed thoroughly in sterile tap water by being shaken with beads in screw-cap prescription bottles. Three dilutions of the washings were made, and each dilution was plated in quintuplicate on two different media. While efforts were directed chiefly toward a qualitative analysis of the flora, quantitative analysis was roughly made where possible.

The molds were recovered from Waksman's (*8*) acid-glucose agar. A modified sodium albuminate agar, used in attempts to recover *Pseudomonas fluorescens* and *Achromobacter radiobacter*, was compounded as follows: Agar, 12.5 g; mannitol, 5 g; glucose, 0.5 g; dipotassium phosphate (K_2HPO_4), 0.5 g; magnesium sulphate ($MgSO_4$), 0.2 g; ferric sulphate ($Fe_2(SO_4)_3$), trace; egg albumen, 0.25 g; peptone, 2.0 g; yeast extract, 25 cc; and distilled water, 975 cc.

After 24 and 48 hours' incubation at 28° C., the plates were studied for the occurrence of *Pseudomonas fluorescens;* after 4 days, for *Achromobacter radiobacter* and the molds. Any colony suspected of being from an inoculated organism was picked to a suitable medium (Waksman's medium for molds; albuminate medium for bacteria), purified, and identified by the usual routine morphological and cultural study. Agglutination reaction in immune sera was used as an aid in identifying *Ach. radiobacter.*

RECOVERY OF ORGANISMS FROM INOCULATED AND NONINOCULATED PLANTS

Throughout the investigation, the recovery of *Achromobacter radiobacter* and *Pseudomonas fluorescens* was considered uncertain. In the case of *Ach. radiobacter* a large number of colonies were isolated, purified, and studied as to fermentation characteristics, physiological tests, and reactions to known *Ach. radiobacter* antiserum. Except in a few cases the results were negative or doubtful. The agglutination reaction, which was considered the most reliable test, eliminated a majority of suspected cultures. Those cases considered positive did not agglutinate to full titer. Also, it has been almost impossible to establish with certainty that many of the *Ps. fluorescens* isolations were actually reisolations of the inoculated cells, because of the expected distribution of this organism in normal soil.

On the other hand, the results would indicate that the molds which were introduced about the root systems were in many cases established there. These results are summarized in table 1. *Aspergillus luchuensis* was found on both healthy and diseased roots in sufficiently greater numbers where inoculated to validate this conclusion. On the other hand, it was never recovered from soil and roots in areas away from the experimental plots. The recovery in a majority of samples analyzed of a form so consistently absent from soil not inoculated with it and from plants not in contact with inoculation would seem to point to successful establishment during the period of observation.

TABLE 1.—*Recovery of test organisms from inoculated and noninoculated plants*

Test organism used for inoculation, and treatment	Samples studied [1]		Root samples analyzed [2]		Healthy plants [2]		Diseased plants [2]		Samples from treatment [3]—					
									1		2		3	
	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery
Aspergillus luchuensis:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Not inoculated	106	6	86	6	43	0	43	6	22	0	50	2	22	2
Inoculated	16	7	16	7	8	2	8	5	2	2	12	4	2	1
Odds [4]	1 : 150,000		1 : 16,000		1 : 2,000		1 : 375,000		1 : 16,000		1 : 370		1 : 6.5	
Trichoderma lignorum:														
Not inoculated	66	4	66	4	33	2	33	2	12	1	40	2	12	1
Inoculated	64	24	44	18	23	7	21	11	12	4	20	9	12	5
Odds [4]	1 : 150,000		1 : 150,000		1 : 80		1 : 16,000		1 : 80		1 : 16,000		1 : 80	
Penicillium luteum:														
Not inoculated	114	44	94	38	48	23	46	17	22	9	50	22	22	9
Inoculated	16	15	16	15	8	7	8	8	2	2	12	11	2	2
Odds [4]	1 : 16,000		1 : 16,000		1 : 20		1 : 2,000		1 : 6.5		1 : 370		1 : 6.5	

[1] Samples included soil and roots, diseased and healthy, single and composite.
[2] Single and composite, from all 3 treatments.
[3] Comparison of recovery from various treatments, both healthy and diseased plants, single and composite samples, from lateral roots only.
[4] Odds against random distribution (7).

Trichoderma lignorum seems to have been established on both healthy and diseased roots in treatments 2 and 3. From the more or less consistent recovery of this form and its equally noticeable absence from plants not inoculated, it would appear that *Trichoderma* has been recoverable over a period of months from plots in contact with inoculum.

Penicillium luteum, although found in normal Texas soil, which complicates interpretation, was recovered in an appreciably greater percentage of cases where inoculated than where it was not introduced. This was especially noticeable on diseased plants and in treatment 2. Although sporadic occurrence is frequent, the evidence herein presented points to a fairly consistent occurrence of *P. luteum* where inoculated.

No detailed study of the spread of molds through soil was made. The findings presented confirm those of Garrett (*3*) in showing that molds are capable of following the surfaces of plant roots as they spread through the soil.

DISTRIBUTION OF INTRODUCED ORGANISMS OVER ROOT SYSTEMS OF INDIVIDUAL PLANTS

The consistent recovery of *Aspergillus luchuensis* and *Trichoderma lignorum* from growing cotton roots suggested the need for further investigation to determine how much of the root system is infected following seed and seedling inoculation. The following procedure was designed to demonstrate the distribution of the introduced organisms over the root systems of the inoculated plants.

SAMPLING

The soil in which two plants, one healthy and one diseased, stood side by side in the same row, was excavated so that the root systems were exposed. A sketch of the root system was drawn to scale, and approximately 10 samples were taken from each root system; each sample was given a number corresponding to a number on the sketch. Samples were 3-inch portions taken from the tip, middle, and base of lateral roots and taproots. Each sample was placed in a tightly stoppered, sterile test tube, and refrigerated until analyzed.

A pair of root systems, one each from a healthy and a diseased plant, were excavated in each of the following locations:

Treatment 2, *Aspergillus luchuensis* rows.
Treatment 2, *Pseudomonas fluorescens* rows.
Treatment 2, *Penicillium luteum* rows.
Treatment 2, *Achromobacter radiobacter* rows.
Treatment 2, *Trichoderma lignorum* rows.
Treatment 1, *Aspergillus luchuensis* rows.
Treatment 1, *Trichoderma lignorum* rows.
Field infested with root rot, 200 feet from the experimental plot.

PLATING

Plates of Waksman's (*8*) and Jordan's (*4*) selective media for *Ps. fluorescens*, and yeast water-mannitol-Congo red agar, selective for *Ach. radiobacter* (*2*), were poured and the agar surfaces thoroughly dried. The root samples were cut into three equal parts and placed respectively on the three media, being pressed lightly to hold them firmly on the surface of the medium. The plates were incubated at 28° C., and daily observations were made.

RESULTS

In treatment 1, *Trichoderma lignorum* was recovered where inoculated in 3 of the 20 samples from two plants studied, twice on healthy roots and once on diseased roots. These results are summarized in table 2. On 20 samples from two plants not inoculated, the organism was never found. In the same treatment, *Aspergillus luchuensis* was

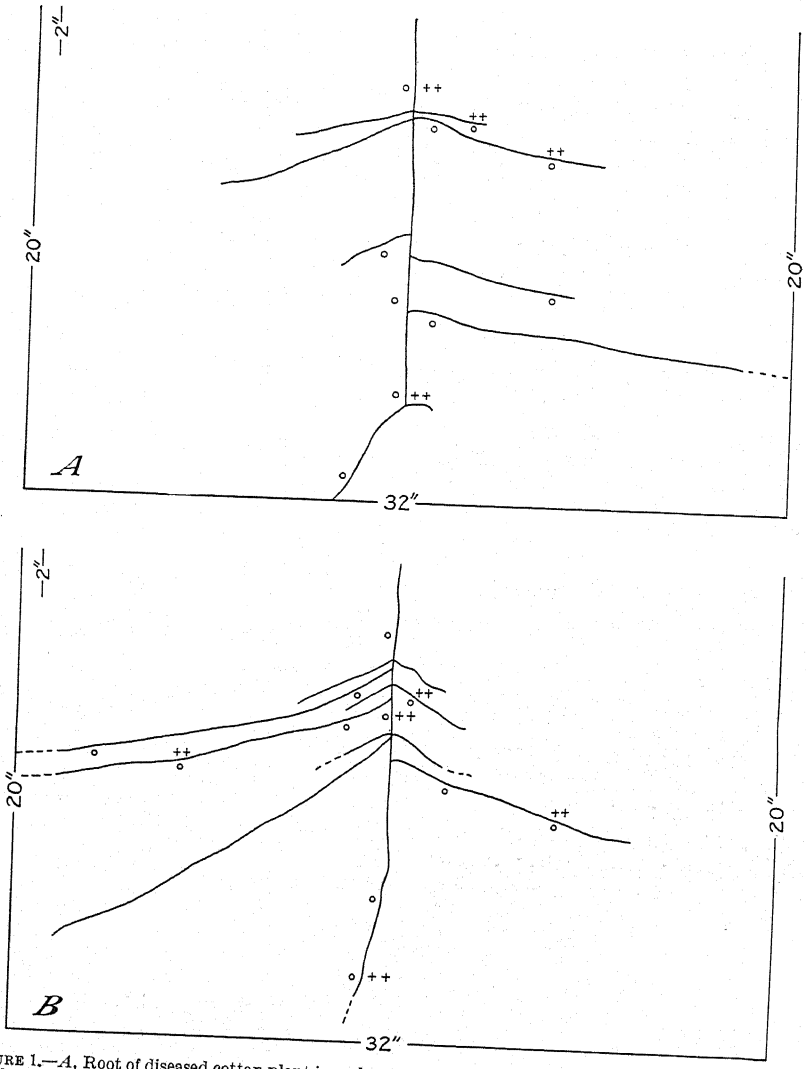


FIGURE 1.—*A*, Root of diseased cotton plant inoculated with *Aspergillus luchuensis;* treatment 1. *B*, Root of healthy cotton plant inoculated with *A. luchuensis;* treatment 2. o. Locations sampled; ++, *A. luchuensis* recovered.

isolated six times from the 20 samples taken from two inoculated plants, but it was not found on plants not receiving inoculum.

In treatment 2, *Trichoderma* was recovered from 8 samples of the 19 taken from two plants inoculated with *Trichoderma*, and twice on 80 samples from eight plants not inoculated. *Aspergillus* was isolated

TABLE 2.—*Summary of results*

Location, treatment, and condition of plants	Cases of recovery of—	
	Trichoderma lignorum	*Aspergillus luchuensis*
Treatment 1, rows inoculated with—		
Trichoderma lignorum:	*Number*	*Number*
Healthy	2 out of 10	0 out of 10
Diseased	1 out of 10	0 out of 10
Total	3 out of 20	0 out of 20
Aspergillus luchuensis:		
Healthy	0 out of 10	3 out of 10
Diseased	0 out of 10	3 out of 10
Total	0 out of 20	6 out of 20
Treatment 2, rows inoculated with—		
Trichoderma lignorum:		
Healthy	5 out of 10	0 out of 10
Diseased	3 out of 9	0 out of 10
Total	8 out of 19	0 out of 20
Aspergillus luchuensis:		
Healthy	0 out of 10	5 out of 10
Diseased	0 out of 10	2 out of 10
Total	0 out of 20	7 out of 20
Penicillium luteum:		
Healthy	1 out of 10	0 out of 10
Diseased	0 out of 10	0 out of 10
Total	1 out of 20	0 out of 20
Pseudomonas fluorescens:		
Healthy	0 out of 10	0 out of 10
Diseased	0 out of 10	0 out of 10
Total	0 out of 20	0 out of 20
Achromobacter radiobacter:		
Healthy	0 out of 10	0 out of 10
Diseased	1 out of 10	0 out of 10
Total	1 out of 20	0 out of 20
Field 200 feet from experimental plot:		
Healthy	0 out of 10	1 out of 10
Diseased	0 out of 10	0 out of 10
Total	0 out of 20	1 out of 20
Total:		
Trichoderma lignorum inoculated rows:		
Healthy	7 out of 20	----------
Diseased	4 out of 19	----------
Total	11 out of 39	----------
Trichoderma lignorum noninoculated rows:		
Healthy	1 out of 60	----------
Diseased	1 out of 60	----------
Total	2 out of 120	----------
Odds against random distribution	1:200,000	----------
Total:		
Aspergillus luchuensis inoculated rows:		
Healthy	----------	8 out of 20
Diseased	----------	5 out of 20
Total	----------	13 out of 40
Aspergillus luchuensis noninoculated rows:		
Healthy	----------	1 out of 60
Diseased	----------	0 out of 60
Total	----------	1 out of 120
Odds against random distribution	----------	1:200,000

from 7 of the 20 samples from two *Aspergillus*-inoculated plants, and was not found in 80 samples from eight plants not in contact with inoculum. These results would seem to confirm the conclusion that the molds *Trichoderma lignorum* and *Aspergillus luchuensis* may be

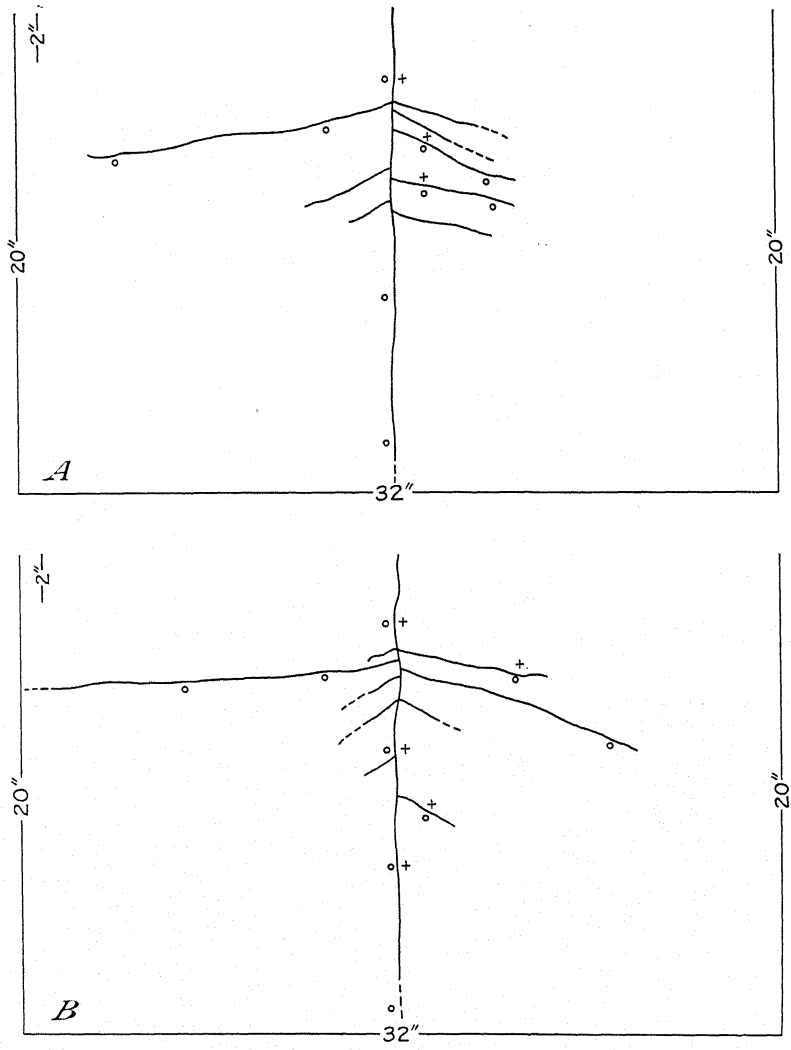


FIGURE 2.—*A*, Root of diseased cotton plant inoculated with *Trichoderma lignorum;* treatment 2. *B*, Root of healthy cotton plant inoculated with *T. lignorum;* treatment 2. o. Locations sampled; +, *T. lignorum* recovered.

found on the living root systems and can be recovered from inoculated plants in from 6 weeks to 4 months after seed and seedling inoculation.

Penicillium luteum was isolated in a large number of cases, but it was also found where it had not been introduced. Because of this it is difficult to draw any conclusions concerning its occurrence or distribution. As in the previous part of the investigation, the

recovery of *Achromobacter radiobacter* and *Pseudomonas fluorescens* was uncertain.

Having determined those root samples in which inoculated organisms occurred, it has been possible to indicate, on the diagrams constructed to show sampling locations, the distribution of the inoculated

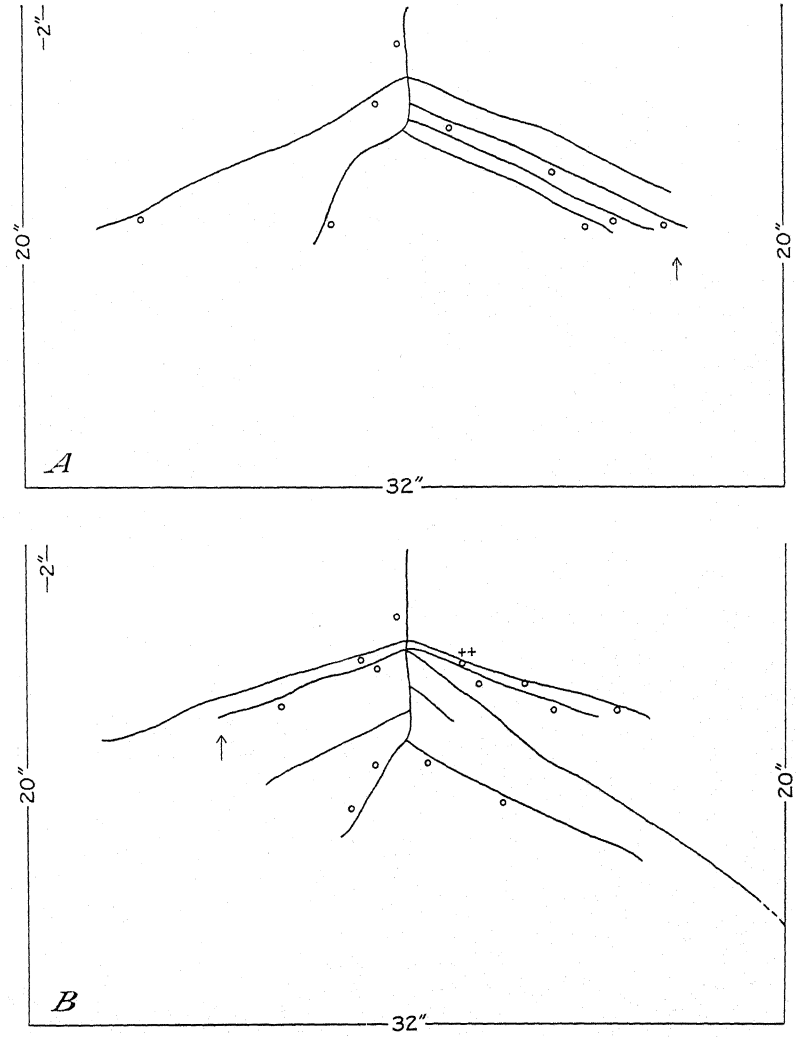


FIGURE 3.—Distribution of *Trichoderma lignorum* and *Aspergillus luchuensis* on root of (*A*) diseased cotton plant and (*B*) healthy plant. o. Locations sampled; ++, *A. luchuensis* recovered. Arrows indicate diseased root of healthy plant adjacent to diseased plant.

forms on a single root system. Six such diagrams are presented in figures 1 to 4.

The diagrams shown in figure 3 are particularly interesting, since they represent growing root systems of a diseased and of a previously healthy plant side by side in the same row and show very strikingly the beginning of infection on one of the lateral roots of a healthy plant

in contact with the infected roots of a diseased plant. No other root on the healthy plant showed symptoms of root rot; and this root showed symptoms only at the tip, where it was in direct contact with the infected root of the diseased plant.

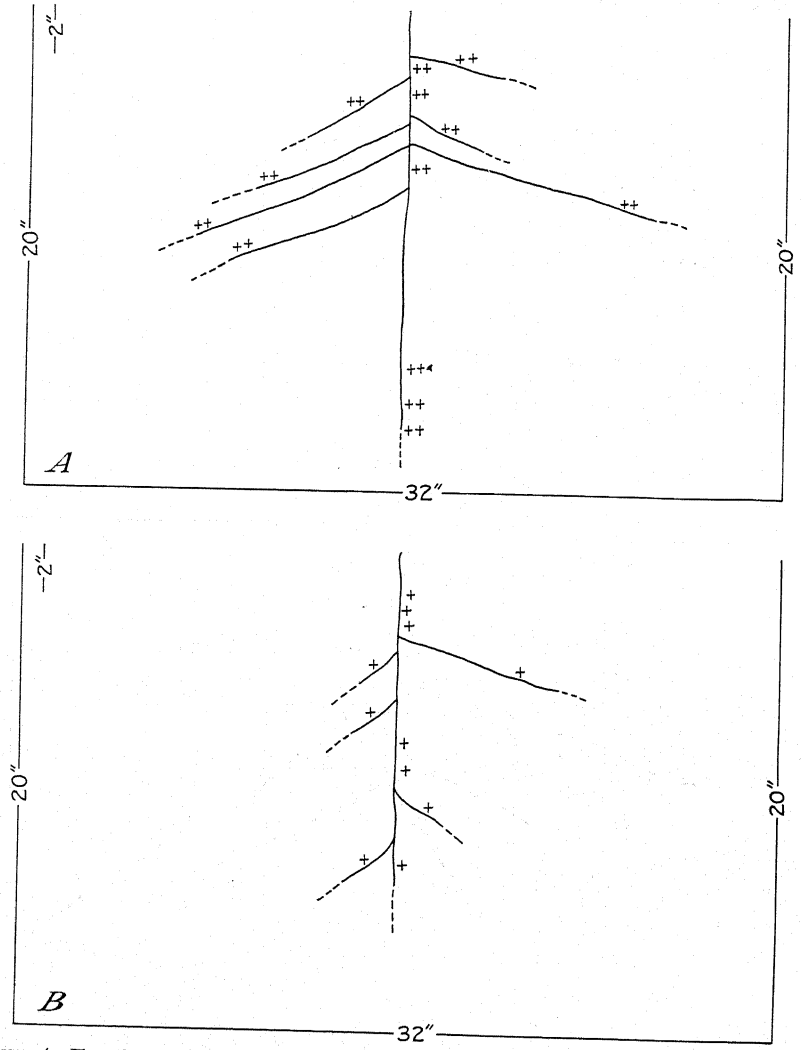


FIGURE 4.—Hypothetical distribution on cotton root of *Aspergillus luchuensis* (++) (*A*) and *Trichoderma lignorum* (+) (*B*).

Shown in figure 4 are all the locations pertaining to *Aspergillus luchuensis* and *Trichoderma lignorum*. This figure represents hypothetical plants with root systems drawn through every point where the respective organisms were found. It is interesting to note that in general *Trichoderma* was recovered only on or near the taproot, while *A. luchuensis* was well distributed over both the taproot and laterals. In view of the fact that the inoculum was placed about the taproot at

a depth of only 4 inches and that these molds have been recovered to a depth of 13 and 17 inches, respectively, for *Trichoderma* and *Aspergillus*, and that the latter organism shows lateral spread from the taproot of some 9 inches, it would seem that the introduced molds have spread on the growing roots, whether or not they have spread through soil free from root material.

SUMMARY

A study of plants from a field infested with *Phymatotrichum omnivorum* (Shear) Duggar has demonstrated that organisms introduced by inoculated cotton seed and by inoculum furnished to the cotton seedling have been established in the rhizosphere of the plant. This conclusion is based upon recovery of the introduced organisms in appreciably greater numbers from inoculated plants than from those not receiving inoculum.

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THE COMPARATIVE VALUE OF COD-LIVER OIL ALONE AND IN COMBINATION WITH IRRADIATED ERGOSTEROL AS A SOURCE OF VITAMIN D FOR CONFINED LAYING HENS [1]

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SURVEY OF LITERATURE

Cod-liver oil has been used for many centuries as an antirachitic agent (*10*).[2] In 1922 McCollum, Simmonds, Shipley, and Park (*24, p. 7*) published an account of a newly discovered factor in cod-liver oil, separate from vitamin A, "which exerts a directive influence on the bone development and enables animals to develop on an inadequate supply of calcium much better than they could otherwise do." Four years later Holmes, Brown, Smith, Treadwell, and Whitelock (*18*) pointed out that cod-liver oil in the diet of laying hens increased egg production and hatchability. That the newly discovered vitamin D was a major factor in their results can be gathered from the work of Hughes, Payne, and Latshaw (*20*), who had previously obtained the same results with ultraviolet irradiation.

In 1924 Steenbock and Black (*31*) found that diets for rats could be made antirachitic and growth promoting by irradiation with a quartz mercury-vapor lamp. In the same year Hess (*13*) and Hess and Weinstock (*15*) activated linseed oil and cottonseed oil by means of irradiation. Either of these activated oils when added to a rickets-producing diet was able to protect rats from rickets. Hess and Weinstock also found that by irradiating cod-liver oil they could enhance its antirachitic value.

Since the unsaponifiable portion of fat contains a large quantity of cholesterol, for a time many research workers considered cholesterol as the precursor of vitamin D. However, Heilbron, Kamm, and Morton (*12*), Hess and Windaus (*16*), and Bills, Honeywell, and MacNair (*6*) demonstrated that in the nutrition of rats, the vitamin D precursor is ergosterol, which is present as a minute impurity in the cholesterol. From this irradiated sterol a crystalline "synthetic" vitamin D was finally isolated. Dissolved in corn oil, irradiated ergosterol is being used extensively as a source of vitamin D.

In the nutrition of rats, Russell, Taylor, and Wilcox (*30*) found that irradiated ergosterol was as efficacious in preventing rickets as cod-liver oil when the two were fed in the approximate equivalent rat-unit dosage. However, in human nutrition, De Sanctis and Craig (*8*) and Barnes, Brady, and James (*1*) concluded that irradiated ergosterol was not so satisfactory an agent in the prevention of rickets as cod-liver oil in the approximate equivalent rat-unit dosage. Holmes (*17, p. 214*), after making a comprehensive review of the

[1] Received for publication October 9, 1937; issued March, 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 218.

literature, decided that as an antirachitic agent "either vitamin D as it occurs in cod-liver oil possesses properties not present in irradiated ergosterol, or that the vitamin A or some other substance in cod-liver oil enhances the antirachitic vitamin in the oil * * *." In the bone development and growth of chicks, Mussehl and Ackerson (*27*), Massengale and Bills (*25*), Bethke, Record, and Kennard (*2*), and Massengale and Nussmeier (*26*) also found that activated ergosterol was much less effective than the rat-equivalent quantity of cod-liver oil. Bethke and associates estimated that 15 to 20 times as many rat units of vitamin D per unit of feed, in the form of irradiated ergosterol, is required for normal bone formation, as in the form of cod-liver oil. Finally, in the feeding of mature hens for optimum egg production and hatchability, Branion and Smith (*7*), Russell, Taylor, and Wilcox (*29*), Bethke, Record, Kick, and Kennard (*3*), and Titus and Nestler (*33*) showed that irradiated ergosterol gave poorer results than the rat-equivalent dosage of cod-liver oil.

Titus and Nestler found that cod-liver oil fed at high levels had a markedly deleterious effect on both egg production and hatchability. They suggested that the optimum quantity of cod-liver oil for strictly confined laying hens is between 1 and 2 percent of the diet, and that when irradiated ergosterol is used as a source of vitamin D, 10 to 20 times as much of this vitamin (as measured by assays with rats) must be fed.

Barnes and associates (*1*), in their observations on humans, found that there was no evidence of superiority among a group of children receiving a mixture of cod-liver oil and irradiated ergosterol as compared with a group given cod-liver oil only. Bethke and associates (*2*) concluded from their work with chicks that a mixture of cod-liver oil and irradiated ergosterol is less effective than the rat-equivalent quantity of cod-liver oil only for proper bone calcification.

Holmes and Pigott (*19*) determined on rats the antirachitic activity of six samples representing five brands of irradiated ergosterol 100 D and found that these samples did not possess uniform antirachitic value. A sample of irradiated ergosterol 100 D that possessed the highest activity of those listed had less than 60 times the antirachitic activity of the best cod-liver oil tested.

Rider, Sperti, Goode, and Cassidy (*28, p. 456*), after making a comprehensive review of the literature, suggested that—

> the expected and the actual clinical potencies of irradiated ergosterol may have been accounted for in part by the now overwhelmingly evident fact that irradiation of ergosterol with the full ultraviolet of the quartz mercury arc produces a multiplicity of products. Some of these products are known to be inactive physiologically and others to be toxic without appreciable antirachitic activity.

These workers found that the discrepancies between the activities of antirachitic agents in rats and chickens are to some extent paralleled by similar discrepancies between their activities in rats and children. In the absence of vitamin D the chicken develops rickets in spite of an optimum calcium and phosphorus ratio and content in the diet. To produce rickets in the rat the diet must be definitely unbalanced with respect to these two constituents. Hess, Lewis, and Rivkin (*14, p. 1888*) make the observation that—

> there is an essential difference in the pathogenesis of rickets in the rat and in infants, that the former is regularly brought about simply by a lack of phosphorus in the diet, whereas rickets never comes about in infants as a result of such a deficiency * * *.

When Bills and associates (*6*) in 1928 activated by ultraviolet irradiation the ergosterol that they obtained from cholesterol, they noted that they could also activate the ergosterol-free cholesterol by the same irradiation. The following year Koch, Koch, and Lemon (*22*) and Koch, Koch, and Ragins (*23*) concluded that provitamin D activity is not limited to ergosterol, but that it may be a general property in varying degrees of various sterols or certain forms of these sterols. More recently Waddell (*34*), Koch and Koch (*21*), Hathaway and Lobb (*11*), and Bethke, Record, and Wilder (*4*) found that in chicken nutrition the vitamin D of irradiated, heated, and purified cholesterol has properties resembling more closely the natural vitamin D of cod-liver oil than has the vitamin D of irradiated ergosterol. In 1937, Bills (*5*) pointed out that at least eight forms of vitamin D have been artificially prepared, and at least two forms occur in fish oils.

In view of the fact that the chicken is less sensitive than the rat to the vitamin D produced by irradiating ergosterol, whereas the opposite is true of the vitamin D in cod-liver oil, the question arises: Is a mixture of vitamin D from these two sources more effective in the case of laying hens than the vitamin D from cod-liver oil alone? As a result of an experiment originally planned for a study of the effect of irradiated ergosterol on the transfer of vitamin A to eggs (DeVaney, Titus, and Nestler (*9*)), data have been accumulated which throw some light on this question. The present paper deals with the writer's analysis of these data.

EXPERIMENTAL METHODS AND MATERIALS

The experiment reported in this paper was conducted at the Agricultural Research Center, Beltsville, Md., during the 48-week period beginning August 29, 1933, and ended July 31, 1934. Six pens were used in this study. They were approximately 8 by 11 feet in size and were located on the second floor of a frame building. Ordinary window glass filtered all the sunlight that entered.

In this experiment there were used 108 cross-bred pullets, the progeny of a mating of Rhode Island Red males with Barred Plymouth Rock females. These birds were distributed in the 6 pens at random, 18 to a pen. Two Rhode Island Red cockerels were placed in each pen, but 5 weeks later the poorer one of each pair was removed. To insure uniform fertility the males were transferred from one pen to the next twice a week.

Throughout the experiment the birds were allowed all they would eat of the following all-mash diet:

	Percent
Ground yellow corn	38.5
Pure wheat bran	18.8
Rolled oats	11.6
Alfalfa leaf meal	4.2
Desiccated meat meal	8.0
North Atlantic fish meal	7.0
Dried buttermilk	5.0
Ground limestone	2.9
Special steamed bonemeal	3.0
Anhydrous sodium sulphate	.5
Sodium chloride	.5
Total	100.0

The proximate chemical analysis of this diet was as follows:

	Percent
Moisture	10.0
True protein [1]	19.2
Nonprotein nitrogen compounds	1.7
Ash	10.3
Fat	4.4
Fiber	4.1
Nitrogen-free extract	50.3
Total	100.0

[1] The diet contained 21.5 percent of crude protein.

A phosphorus level of 1.2 percent and a calcium level of 3.0 percent were maintained throughout the experiment.

The following oil mixtures were added to the diet: Mixture of (1) 0.5 percent of corn oil and (2) 1 percent of cod-liver oil for pen 1, 2 percent of cod-liver oil for pen 2, and 8 percent of cod-liver oil for pen 3; and a mixture of (1) 0.5 percent of irradiated ergosterol 160 D and (2) 1 percent of cod-liver oil for pen 1A, 2 percent of cod-liver oil for pen 2A, and 8 percent of cod-liver oil for pen 3A.

The rachitically inert corn oil was used merely to make the fat content of the diets fed in the first three pens correspond with that of the diets fed in the last three pens. On the basis of the conclusion drawn by Titus and Nestler (*33*), 0.5 percent of irradiated ergosterol 160 D may be considered equivalent to 4 percent of cod-liver oil in its vitamin D value to laying hens. Therefore, whereas pens 1, 2, and 3 received 1, 2, and 8 percent of cod-liver oil, respectively, pens 1A, 2A, and 3A received the equivalent of 5, 6, and 12 percent of cod-liver oil, respectively.

The weights of the birds were obtained at the beginning of the experiment and at the end of every 4-week period thereafter. A record was kept of the weight of the feed given to the birds and the weight of the residue at the end of each 4-week period. Four times a day throughout the experiment the birds in the trap nests were removed and the eggs were collected. Every 4 weeks all the sound eggs laid during a period of 10 days were incubated in order to obtain data on fertility and hatchability. During thirty-three 5-day periods, systematically scheduled throughout the year, all eggs were weighed; and during every third 5-day period the eggs, in addition to being weighed, were tested for shell strength by the method of Swenson and James (*32*), as modified by Titus and Nestler (*33*). In this test for eggshell strength a steel ball weighing 3.5282 g was dropped from various heights through a tube on each egg, which was held in place under the end of the tube. During the last half of the experiment studies were made of the weight and thickness of the eggshells, thickness of the membranes of the eggshells, weight and color index of the yolks, and the weight of the whole and thick albumen, from eggs obtained during five 5-day periods 4 weeks apart. The thickness of the eggshells and the thickness of the shell membranes were determined with micrometer calipers immediately after the eggs were broken. The two membranes were measured together. A color scale designed by Dr. Paul F. Sharp, of Cornell University, was used in the study of the color of the egg yolks. The thick albumen was sepa-

rated from the whole albumen by the use of a perforated sieve 4⅝ inches in diameter, having 64 holes per square inch, each hole being 0.081 inch in diameter.

RESULTS AND DISCUSSION

A summary of the data obtained is presented in table 1, and the statistical significance of the differences found in table 1 is presented in table 2. Most of the data were analyzed by variance analysis.

The birds in pen 1, which received 1 percent of cod-liver oil as the source of vitamin D, made significantly greater gains in live weight between August 29, 1933, and February 13, 1934—the date of maximum gains during the entire experiment—than the birds in pens 1A, 2, or 2A. The differences between the gain in live weight of the birds in pen 1 and the gains of the birds in pens 3 and 3A were large but not statistically significant. Likewise, differences between the gains in live weight of birds in any two of the pens other than pen 1 were not statistically significant.

The birds in pen 1 consumed a greater quantity of feed than any other birds. This fact accounts for their greater gains in live weight. The difference in feed consumption was statistically significant in all cases, except between the birds in pen 1 and those in pen 2A. The diets fed in pens 1A, 2, and 2A all caused significantly greater feed consumption than those fed in pens 3 and 3A. There were no statistically significant differences between the quantities of feed consumed by the birds in pens 2 and 3, which received 2 and 8 percent of cod-liver oil, respectively, as the sole source of vitamin D, and the quantities of feed consumed by the birds in pens 2A and 3A, which received corresponding levels of cod-liver oil mixed with irradiated ergosterol.

The hatchability of the eggs from either pen 1, 1A, 2, or 2A was higher than that of the eggs from either pen 3 or 3A, the difference being statistically very significant. There was no statistically significant difference between the hatchability of the eggs from a pen receiving any particular level of cod-liver oil without irradiated ergosterol and that of eggs from a pen receiving a corresponding level of cod-liver oil with irradiated ergosterol.

The production and the total weight of eggs in either pen 1, 1A, 2, or 2A also were greater than the production and the total weight of eggs in either pen 3 or 3A. However, it will be noted from table 2 that the difference between the egg production in either pen 1 or 1A and that in either pen 3 or 3A was not so statistically significant as the difference between the egg production in either pen 2 or 2A and that in either pen 3 or 3A. In the case of both egg production and the total weight of the eggs produced, there was a statistically greater significant difference between either pen 1, 1A, 2, or 2A and pen 3A than between either pen 1, 1A, 2, or 2A and pen 3. Although there was a tendency for cod-liver oil to give better results than a mixture of cod-liver oil and irradiated ergosterol, none of the differences between the results obtained at the same level of cod-liver-oil intake were statistically significant.

The only statistically significant differences found in the data for the average weight of the eggs was between either pen 1 or 1A and either pen 3 or 3A. The weights of the eggs from the former pens were heavier than those of the eggs from the latter pens.

TABLE 1.—*Effect of cod-liver oil at various levels, fed alone or mixed with irradiated ergosterol, on laying hens and their egg production*[1]

DATA THAT SHOW STATISTICALLY SIGNIFICANT DIFFERENCES[2]

Source of vitamin D	Pen no.	Birds at—		Average gain in live weight Aug. 29, 1933, to Feb. 13, 1934[3]	Average feed consumed per bird per 4-week period[4]	Average hatchability of fertile eggs[5]	Average egg production per bird per 308.5 bird days[6]	Total weight of eggs per bird per 313.8 bird days[7]	Average egg weight	Average yolk weight	Average reading of Sharp's yolk color scale
		Beginning of experiment	End of experiment								
		Number	*Number*	*Grams*	*Kilograms*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	
1 percent of cod-liver oil	1	18	11	679.2±53.7	4.27± .10	68.0±4.5	42.11±3.53	7,985±672	58.0±1.0	19.1± .4	62±6
1 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	1A	18	14	510.3±47.0	4.00± .10	64.4±4.0	42.98±3.21	7,860±613	58.1± .9	18.5± .4	57±6
2 percent of cod-liver oil	2	18	14	477.4±47.0	4.01± .10	70.8±3.9	45.38±3.31	8,080±631	56.2± .9	18.4± .4	57±6
2 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	2A	18	12	481.5±47.0	4.05± .10	71.1±3.8	44.93±3.31	7,925±651	55.7±1.0	18.0± .4	54±6
8 percent of cod-liver oil	3	18	12	568.4±48.4	3.48± .10	39.7±4.7	33.91±3.40	5,700±672	53.7±1.0	16.4± .4	41±6
8 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	3A	18	7	590.7±51.8	3.69± .10	36.7±5.7	29.56±3.79	5,075±722	54.8±1.0	16.8± .5	40±8

DATA THAT DO NOT SHOW STATISTICALLY SIGNIFICANT DIFFERENCES [2]

Source of vitamin D	Pen no.	Birds at—		Average loss in live weight Feb. 13, 1934, to July 31, 1934	Average eggshell weight [6]	Average thickness of eggshells	Average strength of eggshells (drop of steel ball)	Average thickness of membrane of eggshells	Average total albumen weight per egg	Average percentage of thick albumen	Average fertility of total eggs set [9]
		Beginning of experiment	End of experiment								
		Number	*Number*	*Grams*	*Grams*	*Millimeters*	*Centimeters*	*Millimeters*	*Grams*	*Percent*	*Percent*
1 percent of cod-liver oil	1	18	11	287.7± 84.0	6.73±0.15	0.319±0.007	15.86±0.38	0.040±0.002	33.6±0.8	60.30±1.35	79.1±6.0
1 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	1A	18	14	307.5± 74.5	6.57± .14	.307± .006	15.85± .33	.043± .002	34.1± .7	61.44±1.21	84.4±5.6
2 percent of cod-liver oil	2	18	14	322.9± 74.5	6.75± .14	.326± .006	16.90± .34	.041± .002	32.8± .7	58.83±1.21	78.7±5.2
2 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	2A	18	12	250.4± 80.4	6.63± .14	.315± .006	16.18± .36	.042± .002	32.2± .7	57.17±1.21	88.7±5.4
8 percent of cod-liver oil	3	18	12	301.3± 80.4	6.47± .14	.318± .006	16.57± .37	.042± .002	32.0± .7	60.79±1.25	88.0±6.8
8 percent of cod-liver oil plus 0.5 per cent of irradiated ergosterol 160 D	3A	18	7	438.6±105.3	6.25± .19	.324± .009	16.44± .39	.046± .003	32.6±1.0	58.52±1.66	87.5±8.1

[1] In this table standard errors, rather than probable errors, are used.
[2] According to Fisher's z test for significance.
[3] Date of maximum gain during the entire experiment.
[4] Average of the average weights of feed consumed.
[5] Weighted average of the percentage of hatchability of the fertile eggs set for the individual birds.
[6] Data for birds laying fewer than 5 eggs during the experiment were eliminated. Rest of data was adjusted by covariance analysis for the number of days each bird lived. The average number of days lived per bird for those birds from which data were used in this analysis was 308.5 out of a possible 336.
[7] Data adjusted by covariance analysis for the number of days each bird lived. The average number of days lived per bird for those birds from which data were used in this analysis was 313.8 out of a possible 336.
[8] Fresh, undried eggshells.
[9] Weighted average of the percentage of fertility of the total eggs set for the individual birds.

TABLE 2.—*Statistical significance of the differences found in table 1*

Sources of vitamin D compared [1]	Pen No.	Degree of significance in the results obtained in the experiment in respect to [2]—							
		Gain in live weight Aug. 29, 1933 to Feb. 13, 1934 [3]	Feed consumed per period of 4 weeks	Hatchability of fertile eggs	Eggs produced per bird	Weight of all eggs produced per bird	Egg weight	Yolk weight	Color of yolks
1 percent cod-liver oil and—	1								
1 percent cod-liver oil+irradiated ergosterol	1A	S	S	N	N	N	N	N	N
2 percent cod-liver oil	2	H	S	N	N	N	N	N	N
2 percent cod-liver oil+irradiated ergosterol	2A	H	N	N	N	N	N	S	N
8 percent cod-liver oil	3	N	H	H	N	S	H	H	H—[4]
8 percent cod-liver oil+irradiated ergosterol	3A	N	H	H	S	H	S	H	S
2 percent cod-liver oil and—	2								
8 percent cod-liver oil	3	N	H	H	S	S	N	H	S
8 percent cod-liver oil+ irradiated ergosterol	3A	N	S	H	H	H	N	S	S—[5]
1 percent cod-liver oil+irradiated ergosterol and—	1A								
8 percent cod-liver oil	3	N	H	H	S—[5]	S	H	H	S
8 percent cod-liver oil+irradiated ergosterol	3A	N	S	H	S	H	H	H	S—[5]
2 percent cod-liver oil+irradiated ergosterol and—	2A								
8 percent cod-liver oil	3	N	H	H	S	S	N	H—[4]	N
8 percent cod-liver oil+irradiated ergosterol	3A	N	H	H	H	H	N	S—[5]	N

[1] The differences are in favor of the first-mentioned source of vitamin D of each comparison.
[2] H=highly significant (odds of at least 99 to 1); S=significant (odds of at least 19 to 1); N=not significant.
[3] Date of maximum gain during the entire experiment.
[4] The odds are slightly less than 99 to 1.
[5] The odds are slightly less than 19 to 1.

The tables show that the birds in pen 1, which received 1 percent of cod-liver oil without irradiated ergosterol, laid eggs with significantly heavier yolks than those in pen 2A, which received 2 percent of cod-liver oil with irradiated ergosterol. The difference in the average weight of the yolks of the eggs from pen 2, which received 2 percent of cod-liver oil without irradiated ergosterol, and those from either pen 3 or 3A, was more significant than the difference in the average weight of the yolks of the eggs from pen 2A and those from either pen 3 or 3A.

The color of the yolks of the eggs from pens 1A, 2A, and 3A had a tendency to be lighter than the yolks of the eggs from pens 1, 2, and 3, but the differences were not statistically significant. Significant differences existed between the color of yolks obtained from either pen 1, 1A, or 2 and either pens 3 or 3A. The difference in yolk color between the eggs from pen 2A and either pen 3 or 3A was not statistically significant.

None of the differences in the loss of live weight of the birds, the weight, thickness, or strength of eggshells, the thickness of the shell membranes, the weight of the whole albumen, the percentage of the thick albumen, and the percentage of the fertility of the eggs, were statistically significant.

The data presented in this paper substantiate the conclusion drawn from previous work of Titus and Nestler (*33*) that excessive percentages of cod-liver oil in the diet of confined laying hens have a markedly deleterious effect on both egg production and hatchability. In the present work 8 percent of cod-liver oil in the diet of laying hens considerably lowered egg production, hatchability, average weight of eggs, total weight of eggs produced, weight of egg yolks, and weight of feed consumed. It also caused the yolks to have a lighter color.

A mixture of one-half percent of irradiated ergosterol 160 D, a quantity about four times that considered by Titus and Nestler (*33*) as necessary for laying birds, and 1, 2, or 8 percent of cod-liver oil, gave practically the same results as the same percentages of cod-liver oil when fed alone. This fact harmonizes with the observations of Barnes, Brady, and James (*1*) on the lack of effectiveness of a mixture of cod-liver oil and irradiated ergosterol in human nutrition, and those of Bethke, Record, and Kennard (*2*) on the lack of effectiveness of such a mixture for proper bone calcification in chicks.

SUMMARY

An experiment was carried on at the Agricultural Research Center at Beltsville, Md., in 1933–34, to compare the efficacy of cod-liver oil fed alone with that obtained by mixing it with irradiated ergosterol, as a source of vitamin D for confined laying hens. Six pens of cross-bred pullets, the progeny of a mating of Rhode Island Red males with Barred Plymouth Rock females, were used. The birds were kept in strict confinement without access to direct sunlight. These six pens received the following vitamin D supplement: 1, 2, and 8 percent of cod-liver oil in the first three pens, and 1, 2, and 8 percent of cod-liver oil supplemented in each case by 0.5 percent of irradiated ergosterol 160 D in the last three pens. A phosphorus level of 1.2 percent and a calcium level of 3.0 percent were maintained throughout the experiment.

A mixture of 0.5 percent of irradiated ergosterol 160 D with 1, 2, and 8 percent, respectively, of cod-liver oil in the diet of laying hens showed no superiority over the same quantities of cod-liver oil without the addition of irradiated ergosterol.

The relatively high levels of vitamin D produced by combining cod-liver oil at the 1- and 2-percent levels with about four times the quantity of irradiated ergosterol necessary for laying chickens produced no deleterious effects on the hens or their eggs.

Cod-liver oil at the 8-percent level, whether supplemented with irradiated ergosterol or not, had a markedly deleterious effect on the production, average weight, total weight, and hatchability of the eggs; weight of the yolks; and the consumption of feed. It also caused the color of the yolks to be much lighter than those from birds receiving lower levels of cod-liver oil. However, it had no appreciable effect on the other items studied.

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RESISTANCE OF CHICKEN EMBRYOS TO MECHANICAL DISTURBANCES [1]

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INTRODUCTION

The effect of mechanical shock on eggs during incubation has long been a matter of speculation. The idea is prevalent that a sudden jar or vibrations of the earth such as are caused by earthquakes, dynamite explosions, claps of thunder, or even the passing of trains, affect hatchability. Little or no concrete evidence has been presented to support or to disprove this idea although reliable information would be valuable from a practical, as well as a scientific, standpoint. If developing embryos are more easily killed at one age than another, the hatcheryman could avoid unnecessary handling of the eggs at a critical time, and thereby increase the chances for improving the hatch.

The very extensive literature on the susceptibility of the embryo and its parts to various external agents has been summarized by Hyman.[2] Most of this literature has to do with the origin of terata and contains the results of qualitative experiments with very young embryos or with eggs subjected to various external forces prior to incubation. The opinion is generally accepted that all harmful agents produce similar effects when applied at similar times to the whole embryo and that they affect those parts of the embryo that are developing most rapidly. Since development in earlier stages usually proceeds more rapidly than in later stages, it is generally held that the younger embryos will be most affected by a given force. However, quantitative data on the susceptibility of the embryo at successive stages of incubation and data for later stages are very meager. In this paper quantitative data are presented showing the resistance of the embryo at different stages of development to various forces applied externally to the egg.

MATERIALS AND METHODS

Eggs obtained from a flock of White Leghorns at the National Agricultural Research Center, Beltsville, Md., in 1934, were used for these experiments. The eggs were carefully selected to eliminate, as far as possible, variations due to the breeding and the management of the adult birds and the storage conditions of the eggs previous to incubation.

All the experimental eggs, including the controls, were incubated in a force-draft cabinet-type machine operated at 99.9° F. with a wet-bulb reading of 85°. The eggs were racked in the trays, large end up, and were turned four times daily.

[1] Received for publication September 24, 1937; issued March, 1938.

[2] HYMAN, L. H. THE METABOLIC GRADIENTS OF VERTEBRATE EMBRYOS. III. THE CHICK. Biol. Bull. 52: 1-38, illus. 1927.

Susceptibility of the embryos, at different stages of development, to mechanical shock was tested by applying force to the egg in different ways: (1) By placing eggs in a shaking machine, (2) by centrifuging, (3) by striking the eggs against the operator's hand, and (4) by placing them near blasts of dynamite. In order that only strong embryos would be used, the eggs were removed from the incubator and candled just prior to treatment by one of the foregoing methods. After treatment they were again placed in the machine, where they were left for 3 days before they were again removed and candled. All eggs containing dead embryos were broken and the embryos classified as to time of death.

EXPERIMENTAL RESULTS

EFFECT OF SHAKING

Each day during incubation, two groups of eggs containing embryos of known ages were placed in a mechanical shaking machine and treated for 7 minutes. This machine was driven by an electric motor

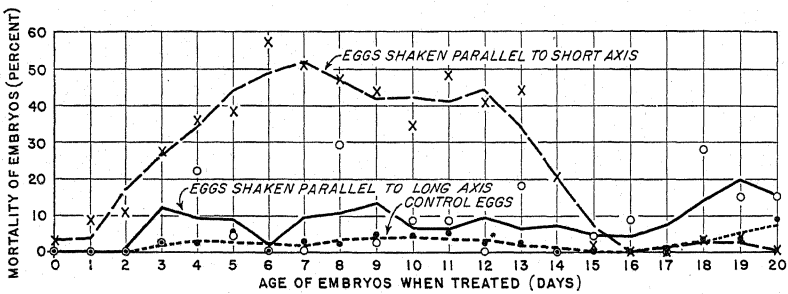


FIGURE 1.—Mortality among embryos in eggs shaken daily for 7 minutes in a mechanical shaker.

at the rate of 229 oscillations per minute through a distance of 3 inches. One group of eggs was so placed in the machine that they were shaken in a direction parallel to the short axis of the egg. The second group was so placed that the direction of force was exerted parallel to the long axis of the egg. During the entire incubation period the test involved 1,252 eggs in the first group, 708 eggs in the second group, and 350 eggs in the control group. The percentage of mortality, showing the effect of this particular type of shock on the embryos at various stages of development, is shown in figure 1

Each point was obtained by dividing the number of deaths which occurred within 72 hours after treatment by the number of live embryos treated. The curves have been smoothed by the three-point method of moving averages. The figure shows that eggs shaken parallel to the short axis of the egg were affected more severely than those shaken parallel to the long axis. In both groups, affected embryos usually died within 72 hours after treatment as a result of rupture of the yolk sac or hemorrhage caused by disruption of the chorio-allantois from the overlying shell membrane. There was little or no delayed mortality and no apparent teratogenic response to treatment at any point in the incubation period. In the eggs shaken parallel to the short axis, mortality attributable to treatment increased rapidly to the fourth day, where it remained within a range

of 30 to 50 percent most of the time until the thirteenth day and then rapidly decreased to zero. In the other treated group, however, the percentage of mortality was rather constant during this period.

EFFECT OF CENTRIFUGING

During each day of the incubation period, two groups of eggs containing embryos of known ages were placed in a centrifuge. One group of eggs, totaling 1,263 at the beginning of this phase of the experiment, was placed in the centrifuge cups blunt end foremost so that the direction of force would be exerted against the large end of the eggs. The second group of eggs, numbering 1,304, was placed pointed end foremost in the centrifuge cups. The eggs were centrifuged for 1 minute, during which time the maximum force exerted on a 2-ounce egg was 1,732 dynes and the average force 710 dynes. After treatment the eggs were removed from the centrifuge and replaced in the incubator, where they remained for 3 days before they were candled and eggs containing dead embryos removed. In

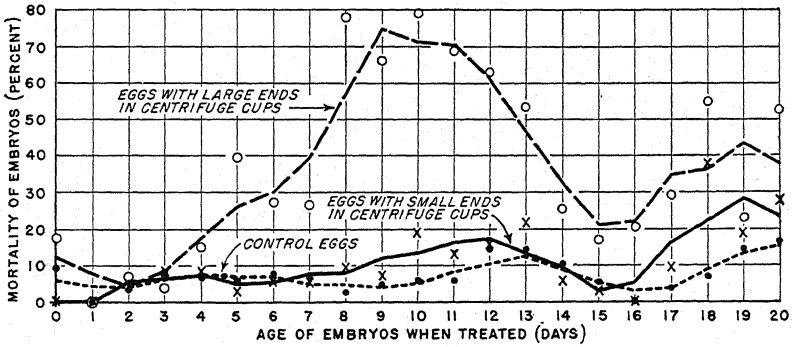


FIGURE 2.—Mortality among embryos in eggs centrifuged for 1 minute.

this phase of the experiment, 850 eggs were used in the control group. The percentage of mortality attributable to treatment on successive days of incubation is shown in figure 2. As in the case of eggs treated in the mechanical shaker, the percentage of mortality was calculated from the number of deaths occurring during a period of 72 hours after treatment. The mortality curves shown in figure 2 have been smoothed by the three-point method of moving averages. The curves are similar to those of eggs that were shaken, in that mortality was much heavier in one of the groups and the heaviest mortality occurred approximately at the same period of incubation.

The effect of treatment in neither group placed in the centrifuge was apparent during the first 3 days of incubation. After that time there was a sharp rise in mortality among embryos in eggs on which force was exerted on the blunt end, the trend line reaching a maximum of 74.0 percent on the ninth day of incubation, gradually receding until it reached 20.5 percent on the fifteenth day, then again rising to an average of about 34.4 percent for the remainder of the incubation period. This same general trend is noticeable but to a far less degree in the group centrifuged with the force exerted on the small end of the egg.

The major cause of death among embryos of both centrifuged groups was found to be broken yolk sacs and ruptured blood vessels of the chorion and the allantois. In these groups of eggs, and especially in the group centrifuged with the large end in the cup, tremulous air cells were produced. Those embryos that survived the treatment showed no ill effects, and in most cases the air cell became fixed again when the chorion and allantois came in contact with the shell membrane. It appears that tremulous air cells are a result of adverse conditions affecting eggs and causing high mortality of the contained embryos and are not the direct cause.

EFFECT OF JARRING

Susceptibility of embryos to mechanical shock was tested by jarring eggs at successive stages of incubation. Each day a group of eggs containing live embryos was removed from the incubator and subdivided into two groups. The blunt ends of one lot of eggs were

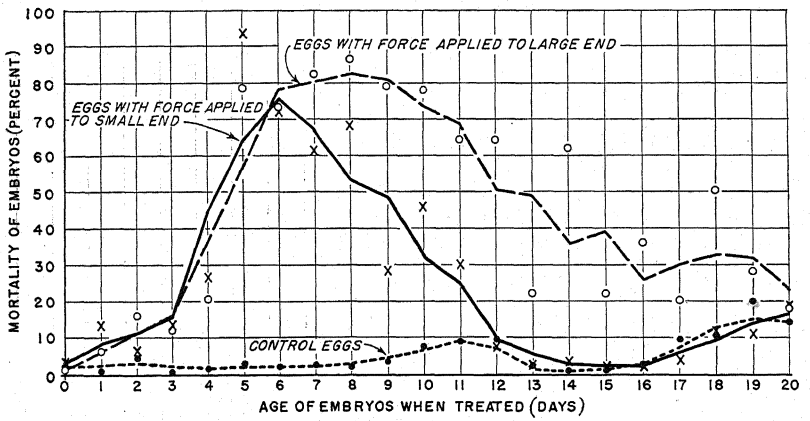


FIGURE 3.—Mortality among embryos in eggs which were jarred against operator's hand.

struck three times against the palm of the operator's hand. The other group was treated in the same manner except that the pointed ends were struck against the operator's hand. In many cases the force was sufficient, especially in the group struck on the blunt end, to produce tremulous air cells in the eggs. During the entire experiment the test involved 1,020 eggs in the first group, 990 in the second group, and 1,163 in the control group. The mortality resulting from the different methods of treatment is shown in figure 3. As in the case of eggs treated in the centrifuge, the points shown on the graph are the percentages of embryos that were killed within 72 hours after treatment. The curves shown have been smoothed by the three-point method of moving averages. It will be noted that the mortality was heavier in the group of embryos in which force was applied on the large end of the egg. This finding is in agreement with that of Knox and Olsen,[3] who found that eggs, when struck on the large end before incubation, failed to hatch as well as either the controls or the group struck on the small end of the egg.

[3] KNOX, C. W., and OLSEN, M. W. THE EFFECT OF TREMULOUS AIR CELLS UPON THE HATCHABILITY OF EGGS. Poultry Sci. 15: 345-348. 1936.

EFFECT OF EXPOSURE TO DYNAMITE BLASTS

Groups of eggs containing embryos of the ages shown in figure 4 were placed near areas where tree stumps were being blasted. The charges of dynamite placed under the stumps ranged from 3 to 28 sticks of 20-percent gelatin dynamite. In this phase of the experiment, 755 eggs were subjected to this type of shock and 623 were used as controls. The former were candled just prior to treatment and then racked, small end uppermost, in an incubator tray and carried to the area where the blasting was being done. The eggs, with the exception of the groups treated on the eighth, ninth, and fourteenth days, were placed within 5 feet of a stump to be blasted and the tray tilted in such a position that the force of the concussion would be exerted on the large end of the egg. To insure against damage from falling objects, the tray of eggs was covered with an empty incubator tray and then was partly buried in the red clay soil. The treatment was so severe that in many cases 20 to 30 percent of the shells of the treated eggs were broken.

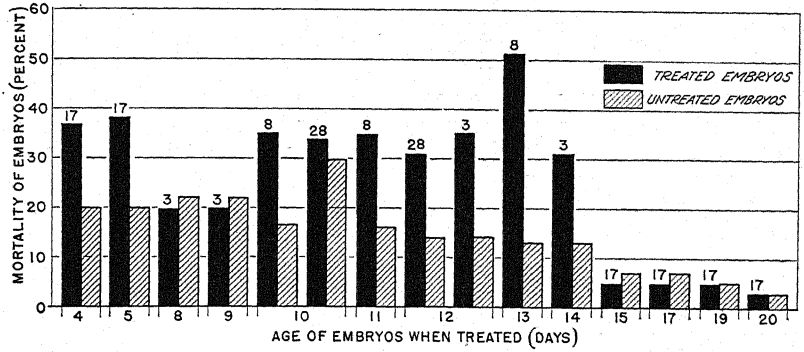


FIGURE 4.—Mortality among embryos in eggs subjected to shock from various sized charges of 20-percent gelatin dynamite. (Numbers at top of bars indicate number of half-pound sticks of dynamite used).

The eggs in the groups treated on the eighth, ninth, and fourteenth days of incubation were racked, large end up, in the incubator trays and the tray placed upon the surface of the ground. Directly over the eggs and within 1 foot of them three sticks of 20-percent dynamite were suspended and discharged. This was done to discover whether the shock transmitted through the air to the eggs was as severe as when transmitted through the ground.

After treatment all the eggs were carried back to the laboratory, where they were candled and those having broken shells were removed. The remainder were replaced in the incubator. The maximum time any group of eggs was out of the incubator during treatment was 30 minutes. The percentage of mortality of embryos in eggs that were not broken when subjected to this type of shock is shown in figure 4. The relative size of charges of dynamite used in each treated group is also indicated. Figure 4 shows the same general effect of this type of mechanical shock as was found in eggs which were shaken, jarred, or centrifuged. In the experiment with dynamite, however, the effect of the shock was cumulative and not so noticeable in mortality during the 72 hours following treatment as at the end of

the incubation period. The percentage mortality shown in figure 4, therefore, is the total mortality from time of treatment to end of incubation. However, this figure in general shows the same susceptible period to mechanical shock, with the exception of the 8- and 9-day points, as shown in the other treated groups, that is, from the fourth to the fifteenth day of incubation. Embryos in eggs subjected to shock on or after 15 days of incubation showed little or no effect due to treatment. The shock on the eighth, ninth, and fourteenth days, as previously pointed out, was transmitted to the eggs through the air instead of through the ground as in the case of the other treated groups. The charges of dynamite used to produce the shock at these points were smaller than those used on the other days of incubation. Either or both of these factors may help to explain the apparent discrepancy in the data.

SUMMARY AND CONCLUSIONS

The data presented show that chicken embryos can be killed by mechanical disturbances caused by jarring, centrifuging, or concussion of the air or ground. In addition, it is shown that embryos are more affected by mechanical shock from the fourth to the fifteenth day than at any other period during incubation. Mortality among embryos was caused chiefly by broken yolk sacs and ruptured blood vessels. In many cases, tremulous air cells were produced by the different treatments, especially when the force was applied at the blunt end of the egg, and in such cases mortality was heavy. The tremulous air cells, in eggs where the embryos survived the treatment, became fixed when the allantois attached again to the shell membrane.

From the results of this experiment, it is recommended that hatching eggs during the period from the fourth to fifteenth day be handled as gently as possible and that they be candled on or after the fifteenth day where this is practicable.

THE VITAMIN A VALUE OF COLOSTRUM AND MILK OF FOUR COWS DETERMINED BY THE SINGLE-FEEDING METHOD[1]

By M. M. KRAMER, *associate in food economics and nutrition*, MARGARET D. BAIR, *graduate assistant*, BERNICE L. KUNERTH, *nutrition technician*, and W. H. RIDDELL, *associate dairy husbandman, Kansas Agricultural Experiment Station*

INTRODUCTION

Some years ago colostrum was shown (*2*)[2] to be higher in vitamin A value than milk produced later in lactation. More recently Semb, Baumann, and Steenbock (*5*), using physical-chemical methods, have reported high vitamin A values for colostral butter. Dann (*1*) studied samples from 14 Milking Shorthorn cows, using colorimetric methods and, for the richest samples, biological tests also. He concluded that the colostrum may be 10 to 100 times as rich in vitamin A as later milk from the same cow.

The physical-chemical methods of determining vitamin A and a precursor, β-carotene, which have been developed in recent years, are useful and handy, but the biological methods remain fundamental, as they summarize total vitamin A activity of the vitamin itself and of any precursor or precursors (*6*).

PROCEDURE

Colostrum and milk samples for these experiments were obtained from four cows in the experiment station herd, fed the regular dairy herd ration, which consisted of a fair quality of alfalfa hay (about No. 2 grade), Atlas sorgo silage, and a grain mixture of yellow corn, bran, and cottonseed meal. Two of the cows freshened in late winter and received only the regular ration; the other two freshened in the spring and had access to rye pasture in April and May, for 40 and 58 days, respectively, before freshening (table 1).

Samples for the determinations were collected four times during the first week of lactation, at the end of the second week, and near the end of the fourth week. In this paper the collections made during the first two days were considered as colostrum. Each sample, representing a 24-hour composite, was stored and kept in a frozen condition until fed.

The single-feeding method of Sherman and Todhunter (*7*) was used for biological assay. Young rats of Wistar Institute strain, weighing 38 to 40 grams, were depleted of vitamin A reserves and divided into groups of 10 or more, comparable in weight, sex, and litter. Single feedings of colostrum or milk were given as indicated in table 2 and negative control rats were maintained throughout. Carotene (*4*) (International standard for vitamin A secured through the Bureau of Chemistry and Soils, United States Department of Agri-

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[2] Reference is made by number (italic) to Literature Cited, p. 232.

culture) was dissolved in an edible cottonseed oil and stored in the dark below 0° C. until needed. Portions of this solution, equivalent to 10 micrograms, 17 micrograms, and 34 micrograms of pure β-carotene, were given as single feedings to the positive control animals to provide data for carotene reference curves (fig. 1).

TABLE 1.—*Description, lactation data, and composition of colostrum and milk of cows used in experiments*

Ration, cow number, and description	Day of lactation	Total daily yield of—			Milk content	
		Milk	Fat	Total solids	Fat	Total solids
		Pounds	*Pounds*	*Pounds*	*Percent*	*Percent*
Standard dairy ration:	First	9.5	0.36	1.64	3.8	7.27
	Second	7.4	.29	.94	3.9	12.76
190, 7 years old, fifth calf Feb. 3, 1935, Holstein.	Fourth	37.6	1.84	5.23	4.9	13.91
	Seventh	42.2	2.41	6.14	5.7	14.54
	Fourteenth	47.9	5.25	6.26	4.7	13.06
	Twenty-sixth	44.0	2.02	5.38	4.6	12.23
241, 3 years old, second calf Mar. 4, 1935, Ayrshire.	First	8.0	.41	1.82	5.1	22.76
	Second	13.9	1.24	3.02	9.0	21.92
	Fourth	28.5	1.36	4.29	4.8	15.05
	Seventh	31.5	1.51	4.54	4.8	14.41
	Fourteenth	32.5	1.40	4.32	4.3	13.28
	Twenty-fifth	34.6	1.45	4.53	4.2	13.01
Standard dairy ration plus rye pasture:	First	11.0	.37	2.49	3.4	22.66
	Second	32.0	1.63	6.39	5.1	19.98
220, 7 years old, fifth calf May 8, 1935, Ayrshire.	Sixth	54.7	2.63	9.24	4.8	16.89
	Twelfth	55.8	2.79	7.92	5.0	14.19
	Twenty-fifth	59.2	2.84	7.56	4.8	12.77
471, 6 years old, fourth calf May 26, 1935, Guernsey.	First	5.2	.27	1.05	5.2	20.11
	Second	18.5	.94	2.84	5.1	15.37
	Fourth	31.6	1.42	4.33	4.5	13.69
	Ninth	32.8	1.74	4.89	5.3	14.90
	Sixteenth	33.4	1.57	4.56	4.7	13.65
	Twenty-sixth	32.8	1.38	4.21	4.2	12.84

None of the cows used was a first-lactation heifer (table 1). Dann (*1*) stated that colostrum from heifers tended to be richer in vitamin A than colostrum from cows, and he suggested that the difference may have been due to the fact that the vitamin A reserve of the heifer had not been diminished by previous lactation. The yields of colostrum presented in table 1 are probably low because complete milking before the fourth day is frequently difficult and undesirable; hence the figures given do not represent entire production. Beginning with the fourth day, however, the yields are complete.

EXPERIMENTAL DATA

In table 2 are presented data from biological assay, summarized and checked against the International standard β-carotene. In each group 10 or more rats were started but it was necessary to eliminate some from the summary; for example, the bloody colostrum of cow 220, first and second days, seemed distasteful to the rats and many refused to eat their portions. The sex ratios of the groups, however, remained well balanced. The feeding experiments extended through about 9 months and included rats whose depleted weights varied from

80 to 105 g, the average for the groups ranging from 89 to 96 grams. The areas under the composite curves (fig. 1) have been shown by the originators of the method (7) to be directly proportional to the vitamin A value of the single feeding. The results of feeding carotene at three levels as indicated in table 2 show that this ratio of areas held for animals of the present series. Readings from the three carotene

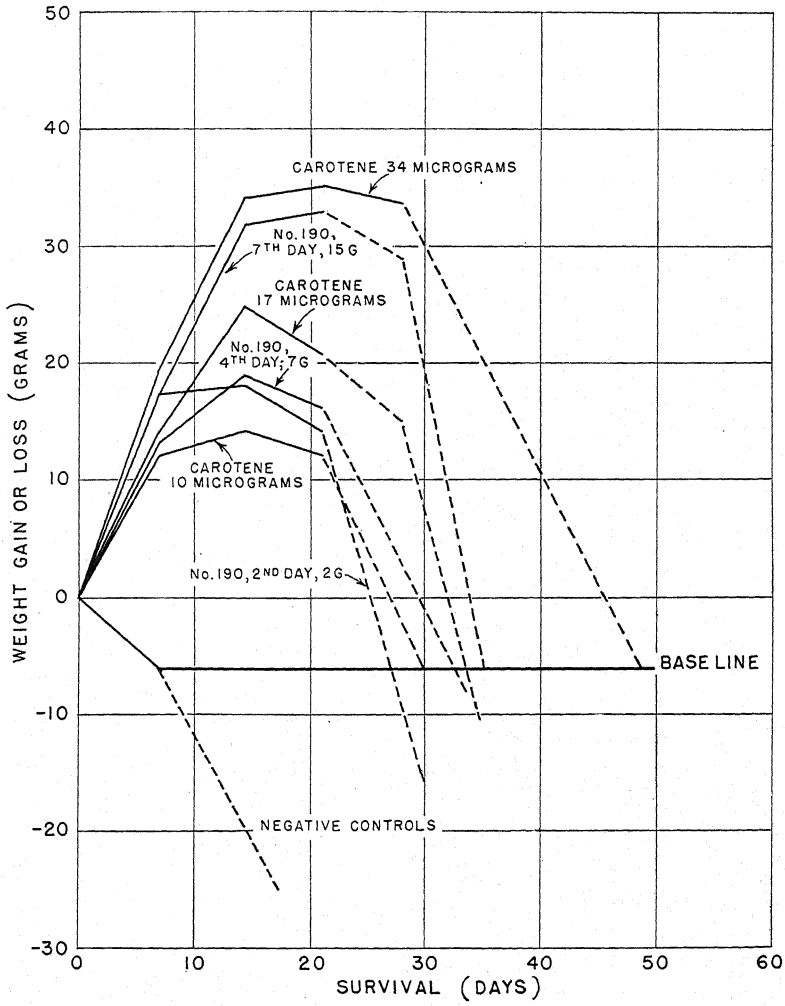


FIGURE 1.—Average gain or loss of rats which had been depleted of body stores of vitamin A and then given a single feeding of colostrum or milk from cow 190 or of carotene in quantities indicated.

reference curves indicate that 2.47 micrograms of pure β-carotene (equivalent to 4.10 International Units) was responsible for an area of 1 square inch. These figures were used to calculate the vitamin A value of 1 g. of each sample in terms of both β-carotene and International Units.

TABLE 2.—*Vitamin A values of colostrum and milk determined by the single-feeding method*

Source of sample		Yield per day		Rats			Figures from composite growth curves			
							Area		Equivalent value per gram in terms of—	
Cow number and ration	Day of lactation [1]	Colostrum or milk	Vitamin A value	Number	Average depleted weight	Single feeding	Total	Per gram fed	β-carotene	Vitamin A
		Grams	*1,000 International Units*		*Grams*	*Grams*	*Square inches*	*Square inches*	*Micrograms*	*International Units*
190 (no pasture supplement).	First [1]	4,309	70	10	90	2	7.90	3.95	9.76	16.20
	Second	3,357	30	7	90	2	4.38	2.19	5.41	8.98
	Fourth	17,055	49	8	91	7	4.90	.70	1.73	2.87
	Seventh	19,142	49	6	93	15	9.28	.62	1.53	2.54
	Fourteenth	21,729	44	8	90	20	9.80	.49	1.21	2.01
	Twenty-sixth	19,958	46	8	91	20	11.16	.56	1.38	2.29
241 (no pasture supplement).	First [1]	3,629	66	8	96	2	8.85	4.43	10.94	18.16
	Second	6,260	128	9	92	2	9.96	4.98	12.30	20.42
	Fourth	12,928	53	10	93	7	6.90	.99	2.45	4.07
	Seventh	14,288	40	7	94	15	10.17	.68	1.68	2.79
	Fourteenth	14,742	25	6	90	20	8.23	.41	1.01	1.68
	Twenty-fifth	15,695	32	5	89	20	10.00	.50	1.24	2.06
220 (supplement of rye pasture).	First [1]	4,990	127	9	96	2	12.40	6.20	15.31	25.41
	Second	14,515	178	6	96	2	5.98	2.99	7.39	12.27
	Sixth	24,812	48	7	92	7	3.28	.47	1.16	1.93
	Twelfth	25,311	46	6	95	15	6.53	.44	1.09	1.81
	Twenty-fifth	26,853	33	7	90	20	5.92	.30	.74	1.23
471 (supplement of rye pasture).	First [1]	2,359	65	8	94	2	13.43	6.72	16.60	27.56
	Second	8,392	162	10	92	2	9.39	4.70	11.61	19.27
	Fourth	14,334	62	10	93	7	7.42	1.06	2.62	4.35
	Ninth	14,878	35	8	91	15	8.60	.57	1.41	2.34
	Sixteenth	15,150	28	9	90	20	8.93	.45	1.11	1.84
	Twenty-sixth	14,878	25	5	90	20	8.25	.41	1.01	1.68
Controls:						*Micrograms*				
β-carotene		-------	-------	11	91	10	[2] 4.14	-------	-------	-------
		-------	-------	23	92	17	[3] 6.73	-------	-------	-------
		-------	-------	9	92	34	[4] 13.67	-------	-------	-------
Negative [4]		-------	-------	25	93	-------	-------	-------	-------	-------

[1] The first sample in each case was collected on the day of freshening.
[2] 1 square inch=2.41 micrograms of pure β-carotene.
[3] 1 square inch=2.48 micrograms of pure β-carotene.
[4] 1 square inch=2.53 micrograms of pure β-carotene.

Average__=2.47 micrograms of pure β-carotene=4.10 International Units.

When comparisons were made on the basis of the vitamin A value per gram (table 2) the secretions of the first or second day of lactation were highest. The two cows receiving pasture supplement produced the richest samples of colostrum tested, containing 25 and 28 International Units of vitamin A per gram. This is consistent with accepted findings (*3*). The vitamin A value per gram of sample dropped rapidly during the first days of lactation (fig. 2, *B*, *D*), and by the end of the first week approached the level maintained during the remainder of the first month. The first samples secured from these two cows were 16 to 20 times as potent as the normal milk at the end of the month.

The richest samples secured from the two cows on the unsupplemented ration contained 16 to 20 International Units per gram. As in the other cases, the vitamin A value decreased rapidly (fig. 2, *A*, *C*). After the first week these cows produced milk containing about 2 International Units per gram. Sherman (*6*) summarized data from many sources and reported the vitamin A value of cow's milk to be 2.92 ± 0.12 International Units per gram.

The rapid drop in vitamin A value per gram of sample as lactation progressed was paralleled by the drop in vitamin A value of the total yield per cow per day (fig. 2). The low vitamin A value for the total yield of cow 190 on the second day of lactation was probably due to

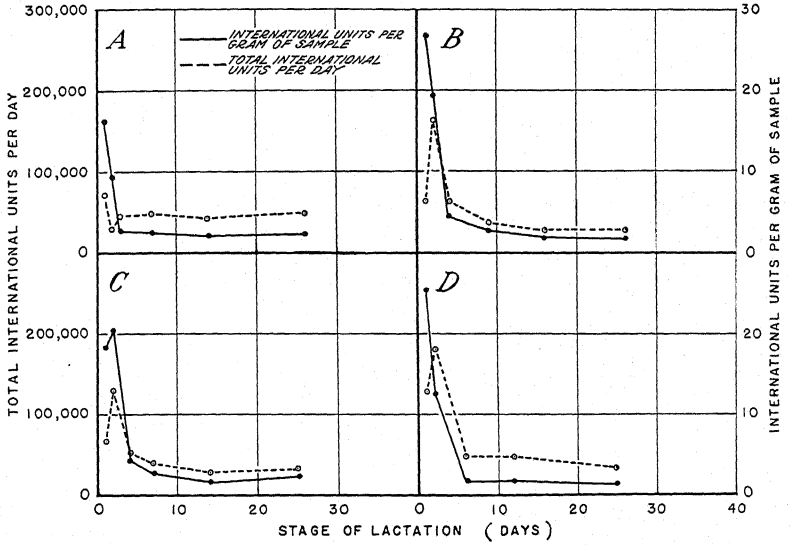


FIGURE 2.—Vitamin A value of colostrum and of milk per gram and per day: *A*, Cow 190, Holstein, no pasture supplement; *B*, cow 471, Guernsey, rye pasture supplement; *C*, cow 241, Ayrshire, no pasture supplement; *D*, cow 220, Ayrshire, rye pasture supplement.

difficulty in obtaining colostrum. Toward the end of the first week, the curve of vitamin A yield per day flattened out for each cow (fig. 2). It is interesting to note that cow 190, a Holstein receiving no pasture, maintained a daily vitamin A output higher than that of any of the other cows.

SUMMARY AND CONCLUSIONS

Samples of colostrum and milk were collected at intervals during the first month of lactation from two cows on the regular dairy herd ration and from two cows on this ration supplemented with rye pasture. The total vitamin A values of the samples were determined by the single-feeding biological method. Reference curves were secured by feeding standard carotene to positive control rats.

The secretions of the first or second day were richest in total vitamin A value, 25 and 28 International Units per gram for the cows receiving rye pasture supplement and 16 and 20 International Units for the others. By the fourth day of lactation there was a considerable drop

in the vitamin A value per gram of sample. In general the vitamin A value of the milk remained much the same from the seventh day to the end of the experiment, approximately 2 International Units per gram. It is interesting to note that one of the cows that received no rye pasture supplement maintained the highest level of vitamin A value per gram of milk throughout the month.

When comparisons are made on the basis of total production of vitamin A per day, the largest total output was found on the first or second day of lactation. Again the cows that received the rye pasture supplement exceeded those that did not, the maximum production of the former being 178,000 and 162,000 International Units and that of the latter 70,000 and 128,000. The rapid drop in vitamin A value per gram of sample as lactation progressed was paralleled by the drop in vitamin A value of the total yield per cow per day. One cow receiving no supplement maintained the highest level of vitamin output during the latter part of the month. In general, the vitamin A value of the total daily production was near the same level from the end of the first week until the end of the month.

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VITAMIN G (RIBOFLAVIN) CONTENT OF COLOSTRUM AND MILK OF COWS DETERMINED BIOLOGICALLY[1]

By M. M. KRAMER, *associate in food economics and nutrition*, INEZ GARDNER, *graduate assistant*, BERNICE L. KUNERTH, *nutrition technician*, and W. H. RIDDELL, *associate dairy husbandman, Kansas Agricultural Experiment Station*

INTRODUCTION

Cow's milk is recognized as a valuable source of vitamin G, but the influence of the individuality of the cow and the stage of lactation on the vitamin G content of milk has not been established. This study deals with the vitamin G (riboflavin) content of milk with special reference to the influence of these two factors.

PROCEDURE

Twenty-four-hour composite samples of colostrum and milk were obtained from four cows (table 1) that received the regular dairy herd winter ration, consisting of a fair quality of alfalfa hay (about No. 2 grade), Atlas sorgo silage, and a grain mixture of yellow corn, bran, and cottonseed meal. All the cows in the experiment freshened in the fall and winter and had no access to pasture in the period immediately preceding or during the collection of samples. The samples were stored at a temperature below 0° C. until used, as Grayson (*6*) [2] has shown that there is no deterioration in the vitamin G of milk during storage if the milk is kept frozen. The first samples (series 1), collected on the first or freshening day and at the end of the first month of lactation gave results indicating superiority of colostrum in vitamin G content. Series 2 was then planned, with an additional collection on the fifth day, to show rate of decline in vitamin G potency of milk. Previous studies in this laboratory had indicated that the vitamin A value of milk at the fifth-day level is approaching normal.

TABLE 1.—*Description, lactation data, and composition of colostrum and milk of cows used in experiments*

Source of sample		Total daily yield of—			Milk content	
Cow no. and description	Day of lactation	Milk	Fat	Solids	Fat	Total solids
		Pounds	*Pounds*	*Pounds*	*Percent*	*Percent*
118, 3 years old, second calf, Holstein.	First	7.0	0.20	0.95	2.9	13.50
	Thirtieth	46.2	1.74	5.51	3.8	11.93
373, 4 years old, third calf, Jersey	First	6.0	----------	1.08	----------	17.92
	Thirtieth	30.1	1.92	4.69	6.4	15.59
197, 6 years old, fourth calf, Holstein.	First	4.4	.24	1.15	5.4	26.17
	Fifth	47.0	2.51	6.81	5.3	14.49
	Thirtieth	63.5	2.82	8.17	4.4	12.87
378, 3 years old, second calf, Jersey	First	6.5	.27	1.13	4.2	17.32
	Fifth	23.9	1.53	3.79	6.4	15.87
	Thirtieth	28.8	1.83	4.77	6.7	16.55

[1] Received for publication July 7, 1937; issued March, 1938. Contribution No. 70, Department of Home Economics, and No. 114, Department of Dairy Husbandry.

[2] Reference is made by number (*italic*) to Literature Cited, p. 237.

The biological method of Bourquin and Sherman (*3*) was used for vitamin G (riboflavin) assay. Extensive investigation of the components of vitamin G (B_2) has aroused concern regarding the validity of methods of biological assay, but Booher, Blodgett, and Page (*2*) and, more recently, Bisbey and Sherman (*1*) have confirmed the accuracy of the Bourquin-Sherman (*3*) method. All rats (Wistar Institute strain) were kept in cages with raised screen floors and checked daily so that no animal consuming excreta would be retained on experiment. The rats of series 1, having been kept on stock diet until 4 weeks of age (*3*), were larger and older than those of series 2. The rats of series 2 were fed the stock diet only until they had reached weights of 38 to 40 grams; this procedure was in accordance with the suggestion of Roscoe (*10*), who used 30- to 40-gram rats, Supplee et al. (*11*), who used animals 20 to 26 days old and preferably 40 to 45 grams in weight, and Prunty and Roscoe (*9*), who used rats weighing 35 to 50 g. Lassen (*7*) also preferred 3 weeks to 4 weeks old rats as they were more uniform in size and varied less in subsequent response. Depleted weights for rats of series 2 were correspondingly lower. Samples to be assayed were fed in daily portions as indicated in table 2. Riboflavin [3] (lactoflavin, PX grade) was fed as a standard for reference, and herd milk was given to another group of rats for comparison. Negative controls were maintained throughout.

TABLE 2.—*Vitamin G (riboflavin) content of colostrum and milk determined biologically*

Source of sample		Yield of milk per day	Rats					Estimated flavin—		Yield of vitamin G per day
Series and cow number	Day of lactation		Number	Average initial weight	Average weight when depleted	Average gain in 8 weeks	Amount of sample fed	Per feeding	Per gram	
		Grams		*Grams*	*Grams*	*Grams*	*Grams*	*Micrograms*	*Micrograms*	*1,000 micrograms*
Series 1:										
118	1st	3,175	9	59	68	30	1	4.5	4.5	14
	30th	20,956	10	56	63	33	3	5.0	1.7	36
373	1st	2,722	10	57	62	30	1	4.5	4.5	12
	30th	13,653	10	51	62	42	3	6.4	2.1	29
Series 2:										
197	1st	1,996	11	39	47	44	1	6.7	6.7	13
	5th	21,319	11	39	50	49	2	7.4	3.7	79
	30th	28,803	10	38	46	47	3	7.1	2.4	69
378	1st	2,948	11	38	48	28	1	4.2	4.2	12
	5th	10,841	11	39	46	43	2	6.5	3.3	36
	30th	13,063	11	39	48	46	3	7.0	2.3	30
Controls:										
Herd milk, winter		----------	14	39	45	40	3	6.0	2.0	--------
Lactoflavin (Labco PX).		----------	9	39	49	35	[1] 5.0	--------	--------	--------
Negative controls		----------	25	39	46	±0	--------	--------	--------	--------

[1] Micrograms.

EXPERIMENTAL DATA

At present it seems desirable to estimate vitamin G as micrograms of riboflavin (table 2) by comparison with the flavin reference curve.

[3] Secured from Bordon Co.

No flattening of this curve occurred to suggest an unknown limiting factor in the vitamin G-free diet.

Judging by the composite growth curves of the rats (fig. 1), 1 g of first day colostrum, 2 g of fifth-day, and 3 g of thirtieth-day milk contained comparable amounts of vitamin G. The richest sample studied was the colostrum produced by cow 197, containing 6.7 micrograms per gram, while the other colostral samples had estimated values of 4.2 and 4.5 micrograms per gram (table 2, fig. 2). The two fifth-day samples were less potent than colostrum produced by the same cows, containing only 3.7 and 3.3 micrograms of flavin per gram. Differences in vitamin G content of samples due to stage of lactation

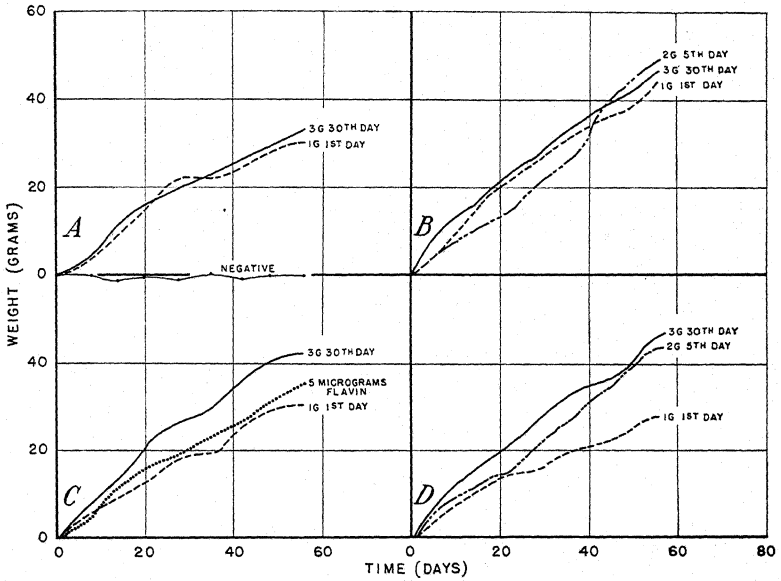


FIGURE 1.—Gain in weight of rats fed colostrum or milk: *A*, From cow No. 118, series 1; *B*, from cow No. 197, series 2; *C*, from cow No. 373, series 1; *D*, from cow No. 378, series 2.

are more pronounced than differences between samples of the four individual cows at the same stage of lactation.

Milk of the thirtieth day was still less potent, containing 1.7 to 2.4 micrograms of flavin per gram, with an average of 2.1, which is similar to 2.0 micrograms, the estimated value of herd milk. The vitamin G content of herd milk was, therefore, about 1,950 micrograms per quart. Other work (*5*) indicates that the flavin content of milk may be 2 to 3 micrograms per milliliter. MacLeod et al. (*8*) concluded that milk from the Walker-Gordon dairy contained 0.3 of a Bourquin-Sherman unit of vitamin G per gram. Day and Darby (*4*) have suggested that a Bourquin-Sherman unit equals 4 micrograms of lactoflavin, a ratio consistent with findings in this laboratory. On this basis, the Walker-Gordon milk contained 1.3 micrograms per gram, while pasteurized milk available in New York City contained 3 micrograms per gram, according to Todhunter (*12*).

Total 24-hour yields of vitamin G for each of the four cows used in the investigation were estimated for the stages of lactation studied

and recorded as thousands of micrograms. The yields of vitamin G from the four cows for the first day of lactation were similar, 12,000 to 14,000 micrograms. These values are probably low because of the inadvisability of complete milking. Data from only two cows were available for the fifth day, and these showed a marked difference (fig. 2). However, it is interesting to note that each cow produced somewhat more vitamin G on the fifth day than on the thirtieth day, with cow 197 higher each time. This cow, a Holstein, excelled in other ways. At the end of the first month of lactation, when she was yielding more than twice as much vitamin G (69,000 micrograms) per day as the mean production of the other cows (32,000 micrograms), she also produced 81 percent more milk, 51 percent more butterfat,

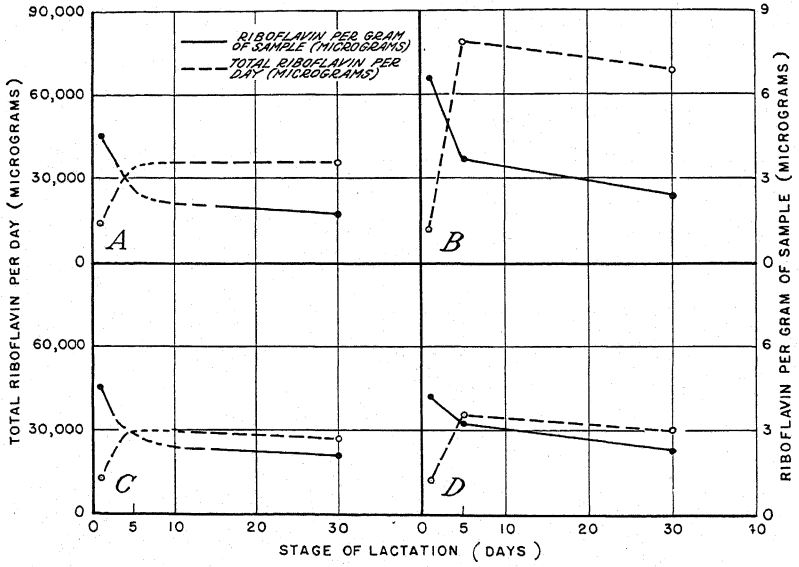


FIGURE 2.—Vitamin G (riboflavin) per gram and per daily yield of colostrum and milk: *A*, Cow No. 118 series 1; *B*, cow No. 197, series 2; *C*, cow No. 373, series 1; *D*, cow No. 378, series 2.

and 43 percent more total solids than the other cows. In contrast to the drop in vitamin G content per gram of milk as lactation progressed, the total daily output of the vitamin per cow increased owing to the increase in the yield of milk. The relationship of content per gram to total yield of flavin is presented graphically in figure 2.

SUMMARY AND CONCLUSIONS

Twenty-four-hour samples of colostrum and milk from two Jersey and two Holstein cows on the first, fifth, and thirtieth days of lactation, together with a sample of herd milk, were tested for vitamin G (riboflavin) content by biological assay. Flavin reference curves were used to estimate the vitamin G content per gram of sample. The vitamin G content of milk from individual cows at the end of 1 month of lactation and also a composite herd sample was estimated as about 2 micrograms per gram, or 1,950 micrograms per quart.

The samples of fifth day milk tested were approximately 50 percent richer in vitamin G than the later milk from the same cows or the regular herd milk. First-day colostrum was richest in vitamin G and was estimated to contain 4.2 to 6.7 micrograms per gram. A gram of colostrum, 2 g of fifth-day milk, and 3 g of milk at the end of the first month of lactation gave approximately the same total gains in weight for the groups of rats fed, indicating a similar vitamin G content.

On the first day of lactation the cows all yielded somewhat more than 12,000 micrograms of vitamin G. The largest total daily outputs were on the fifth day for the cows from which such samples were saved, but these totals approached the yields for the thirtieth day. On the thirtieth day of lactation three cows produced about 32,000 micrograms each, while the other cow, a high milk producer, yielded twice this amount of vitamin G.

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LIFE SPAN AND MORPHOLOGY OF FIRE BLIGHT BACTERIA AS INFLUENCED BY RELATIVE HUMIDITY, TEMPERATURE, AND NUTRITION[1]

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INTRODUCTION

The problem concerning the sources of inoculum for the earliest or primary fire blight on apple and pear trees in the Ozarks of Arkansas remains obscure. Unlike more northern and western fruit-growing areas, where excellent evidence has been found for the overwintering of the pathogen in formerly blighted limbs and twigs and its dissemination from such hold-over blight to blossoms and twigs, no such evidence has been found in the Ozarks (*11, 13, 14*).[2] This should occasion no surprise. The marked differences in climatic conditions, in length of growing season, and in varieties grown must undoubtedly have some influence on the variation of the life of the pathogen. The relative scarcity or absence of fire blight west of the Cascades is well known, in contrast to its prevalence in fruit-growing areas east of this mountain range, and as Heald (*7*) has noted:

* * * sufficient time has elapsed since its arrival in the coast country for it to have become prevalent in western Washington, hence it seems possible that climatic factors are responsible for its absence from this section.

In an effort to discover the sources of inoculum for early spring infections in the Ozark region, various lines of investigation have been pursued, including the possible overwintering of fire blight bacteria within beehives (*13*) and the overwintering of bacterial exudate either on parts of the trees above ground or in the soil (*16*). However, none of the evidence deduced from these studies is conclusive (*13, 16*). The problem is still unsolved, not only for the Ozarks of Arkansas but for the Southern States as a whole, although the use of open-blossom sprays by the writer (*14*) has been based on the assumption that the first spring blight is occasioned mainly by the dissemination of the bacteria by blossom-visiting insects, especially bees.

It is clear, however, that without knowledge of the influence exerted by various combinations of climatic and nutritional factors on the life of this parasite, conclusions relative to the longevity and prevalence of bacteria drawn from such tests as artificial contamination of beehives, attempted isolations from infested hives, and isolations from insect bodies, soil, or diseased tree parts may have very restricted significance and at best be applicable only to local or purely artificial conditions. To gain more definite information on the influence of

[1] Received for publication July 27, 1937; issued April 1938. Research paper No. 483, of the Journal Series, University of Arkansas.

[2] Reference is made by number (italic) to Literature Cited, p. 257.

important climatic factors and to a limited extent of nutritional factors on the life of the organism, investigations reported here were undertaken. A special effort was made to study the behavior of the bacteria in naturally occurring exudate rather than their behavior in pure, artificial cultures, although the latter were used for comparison.

REVIEW OF PREVIOUS WORK

Since a review of the older literature pertaining to the influence of dryness on the longevity of the fire blight organism, *Erwinia amylovora* (Burrill) Winslow et al. (*Bacillus amylovorus*), has been presented previously (*16*), there is no need of duplicating it here. In that paper evidence was presented to show that the bacteria in the form of exudate derived from blighted tissues are very much less sensitive to dry air than had generally been supposed; and that at a controlled relative humidity approaching zero they were found to be alive at the end of a year under laboratory conditions of fluctuating temperatures and reduced light. Likewise, it was reported that exudate kept outdoors in corked vials, under uncontrolled light, temperature, and humidity conditions, yielded dead bacteria in short order, and that the organisms in the form of hardened exudate left in place on a cankered pear trunk exposed to direct sunlight also died within a few days.

Good evidence has very recently been presented by Parker (*10*) confirming the findings relative to the longevity of *Erwinia amylovora* under dry conditions, and adding to our knowledge concerning the combined influence of temperature and humidity. Using temperatures of 31°, 52°, and 70° F. and both very dry conditions, maintained by the use of calcium chloride desiccators, and more moist conditions in open dishes, he found that bacteria derived from exudate lived at least 52 days, the last day of testing, at 31° and 52°, and at least 45 days at 70°, all under dry conditions, while under more moist conditions in open dishes they were shorter lived.

Ark (*1*) has also reported very recently on studies of fire blight bacteria which included the influence of dryness on the life of the bacteria. First he reports very briefly that he "was able to isolate viable bacteria from bacterial exudate kept in a vial in a dry laboratory closet for 2 years and 10 months." Whether these bacteria were tested for pathogenicity is not stated, and one wonders what tests were used to determine if the isolated bacteria actually represented *Erwinia amylovora*. However, he performed two additional experiments involving the influence of dryness, though both were primarily concerned with discovering differences in reaction of different isolates. Using four isolates derived from unlike hosts and from diverse parts of the United States, he grew them in beef-extract agar, suspended the growths in a saline solution, and tested for the influence of dryness by smearing a drop of the bacterial suspensions on cover glasses maintained at different degrees of dryness. The bacteria survived from 18 to 36 hours, the latter period representing the longest utilized. "The degree of humidity seemed to be of no significance as far as the longevity of *E. amylovora* is concerned in this experiment," he concluded. In a second experiment, which also involved artificial culture media, gauze strips suspended in broth cultures were removed after 48 hours of growth, the strips hung in large sterile test tubes, and pieces cut off and cultured from time to time. Two isolates were

found to be nonviable after 6 days, a third after 4 days, and the fourth after 10 days. He reported:

> Both of the experiments were repeated and in each case the same results were obtained. Thus, it is safe to conclude that different degrees of susceptibility to dryness exist among isolates of *Erwinia amylovora* * * *.

Ark makes no comment of the amazing discrepancy in longevity under dry conditions shown in his test of viability of bacterial exudate compared with his tests of pure cultures.

So far as studies on the influence of temperature are concerned, most of these have in the past involved pure cultures, usually maintained in beef-broth or synthetic media in which the standardized procedure of determining maximum, optimum, and minimum temperatures for growth was followed and little attention paid to the life span of the bacteria at the different temperatures. With the exception of the very recent paper by Ark (*1*), these former temperature studies have been reviewed *(14, p. 4)*. Ark followed the same procedure used by previous investigators and obtained comparable results.

With reference to nutritional factors influencing the longevity of this organism, the writer has no knowledge of earlier work. At times it has been assumed that fire blight bacteria grown in beef-broth or nutrient agar will be comparable, morphologically and physiologically, to those growing within apple or pear tissues, and such tests as applying beef-broth or agar cultures to beehives followed by attempts to reisolate the parasite after varying intervals of time from the hive have been used with the assumption that the data obtained would be comparable to those resulting from the transfer of bacteria from a pear or apple blossom to the hive by a honeybee. That differences in nutrition—as exemplified by growth in beef-broth or nutrient agar compared with bacterial growth in natural host tissues, or in nectar—might conceivably yield wholly unlike results in physiological response as well as in morphology had not, to the writer's knowledge, been entertained.

Since part of the investigations herein described concerns differences in morphological response, particularly that which deals with the presence or absence of a capsule, sheath, or slime formation, several investigators report the absence of capsules for this species of bacteria (*5, 8, 19*).

MATERIALS AND METHODS

The bacterial exudate utilized consisted of four collections. Lot 1 was made up of the exudate placed over concentrated sulphuric acid on June 11, 1934, used in earlier work (*16*) and continued in these investigations.

Lot 2 consisted of exudate gathered on May 21 and 28, 1935, from freshly blighted shoots and blossom clusters of Jonathan apple trees located in a commercial orchard near Rogers, Ark. This material was kept in a refrigerator overnight and placed the next day in small, paraffin-covered paper baskets suspended in 200-cc bottles over concentrated sulphuric acid having a specific gravity of 1.835. Most of this exudate when gathered was in the form of small, milky, or light-brown droplets which had not fully hardened and were still quite viscid. In many instances it was found necessary to scrape the epidermal hairs of the young twig, petiole, or pedicel, so that the droplets of exudate as placed in the bottles were often enmeshed in these hairs. No attempt was made to sterilize the bottles or to remove the

exudate from the blighted material under aseptic conditions, since one of the objectives of these investigations was to determine the longevity of fire blight bacteria in the presence of naturally occurring, competing, or antagonistic micro-organisms that might be present in the orchard.

On June 21, after the material had been kept under the same conditions of relative humidity, temperature, and light for a period of 3 to 4 weeks, and had reached the same degree of dryness, as measured by the specific gravity of the sulphuric acid, it was divided and treated as follows: 22 divisions were made to be kept under as many combinations of relative humidity and temperature. To obtain different relative humidities, various concentrations of sulphuric acid-water mixtures were made, the physical table presented by Wilson (*21*) being used as the guide. Specific-gravity determinations used as a check on the theoretical computations indicated that the following relative humidities were obtained: 0, 9.5, 21, and 45 percent. The amount of sulphuric acid or sulphuric acid-water mixture utilized, the placement of the open baskets, the size of the bottles used, the sealing of the rubber stoppers with melted paraffin, all followed the same methods described in the former article (*16*). For the exudate kept at approximately zero relative humidity, six bottles were used, one for each of the following temperatures: 16°, 25°, 30°, 35°, and 40° C., and outdoors (fluctuating temperature). The last bottle was tied in a wire basket and suspended from an upper limb of a large apple tree. The other three relative humidities, in combination with the different temperatures noted, were utilized as in the zero relative humidity, except that insufficiency of exudate did not permit using the outdoor temperatures for all the relative humidities.

Lot 3 of exudate was gathered on May 19, 1936, from freshly blighted Bartlett pear shoots. It was immediately placed over concentrated sulphuric acid, allowed to harden thoroughly, and on June 15, 1936, divided into two parts, one placed over an acid-water mixture yielding an approximate relative humidity of 45 percent, and the other over concentrated sulphuric acid, specific gravity 1.835. They were then transferred outdoors into United States Weather Bureau-type housing.

Lot 4 of exudate was also gathered from freshly blighted Barlett pear shoots on July 30 and August 3 and 14, 1936. It was placed over concentrated sulphuric acid on each date of collection and kept at laboratory temperatures until September 4, 1936. On that date the thoroughly hardened droplets were divided into three parts, placed in bottles containing sulphuric acid-water mixtures which yielded relative humidities of approximately 50, 75, and 90 percent, and were kept at 25° C. for 5 days. They were then taken outdoors and placed in the United States Weather Bureau-type housing.

As previously noted, the bacterial exudate in the different lots was first placed over concentrated sulphuric acid, so that all of it would be brought to approximately the same degree of dryness. It seemed essential to have all the material as uniform as possible relative to the amount of moisture present, prior to its use for experimental purposes.

In order to study the influence of dry air and different temperatures on *Erwinia amylovora* when present within blighted host tissues, freshly diseased twigs and leaf petioles of Jonathan apples were gathered on May 21, 1935, in the same orchard from which exudate was obtained. The day after the material was gathered it was cut

into lengths varying approximately from one-fourth to three-eighths inch, so that it could be accommodated in the small baskets kept over the acid. An effort was made to use only fresh infections; all material that showed signs of hardening of the tissues and last stages of blight development were rejected. However, subsequent tests revealed that some of the material, in addition to harboring *E. amylovora,* was also the seat of nonpathogenic, yellow bacteria—rather common followers of fire blight bacteria within diseased tissues (*12*)—and hence was not representative of strictly fresh infections. Fortunately the number of pieces showing such secondary invasion was not large.

This diseased material was divided into two lots, both placed over concentrated acid, but one was kept in a bottle placed outdoors and suspended from an apple limb in the same manner as described for the bacterial exudate kept outdoors, and the other was kept under laboratory conditions.

The pure cultures of *Erwinia amylovora* used for these tests were made up as follows: A recent isolate which had proved to be highly virulent in artificial infection was replated on nutrient dextrose agar, and after 48 hours incubation, a single vigorous colony was transferred in the form of smears on a plate of the same medium used previously. After 48 hours, a second set of smears was made, again on the same medium, and also transferred to tubes of nutrient dextrose broth. After 48 hours' growth, the smears, together with the underlying medium, were cut into blocks approximately 1 cm square, and immediately placed to dry in open baskets over concentrated acid in corked and sealed bottles. At the same time, one of the tubes of broth with 48 hours' growth was employed for testing the pathogenicity of the isolate. Several healthy, succulent Bartlett pear shoots were injected with the bacterial suspension, and within several days all inoculated shoots showed characteristic blight symptoms, while uninoculated controls remained free.

At intervals following the placement of the different materials in the various relative humidities and temperatures, parts were removed and treated as follows: A droplet of exudate was placed in approximately 0.2 to 0.5 cc of sterile distilled water, resulting in a very heavy bacterial suspension, which was injected with a sterilized hypodermic syringe into healthy succulent Bartlett pear shoots; or cultures were plated out and single-colony transfers used for injecting pear shoots. In the first few series of trials injections were made from single-colony transfers but this was found to be unnecessary because it was discovered that whenever viable *Erwinia amylovora* were present in sufficient numbers to induce infections, direct injections of the original exudate suspended in water were just as reliable in testing the presence of live bacteria as the more cumbersome plating-out, single-colony transfer, and final injection method. Furthermore, in plating out, there is always the danger of losing any relatively slow-growing pathogen. In both cases the final proof for the viability and infectivity of the bacteria was based on the presence or absence of typical fire blight symptoms a few days following the artificial inoculations and on the presence or absence of *E. amylovora* in the infected tissues. If no fresh exudate appeared on the artificially infected tissue, the latter was surface-sterilized, washed in sterile water, and used for a series of poured dilution plates. When typical colonies appeared, single ones were re-isolated and again used for inoculation.

To determine the presence of viable bacteria within diseased tissues kept under the conditions indicated previously, several pieces were removed at different times, soaked and mashed in sterile water, and then used in a similar manner for testing the presence of viable bacteria as in exudate. Likewise, to test the viability of bacteria on the agar sheets (pure cultures), several of these were removed from the bottles at any one test, allowed to form heavy bacterial suspensions, and injected into healthy succulent Bartlett pear shoots.

All inoculations were made on trees growing in a greenhouse, and a sufficient number of uninoculated control shoots were at hand for comparative purposes as each test was undertaken. No infections occurred on the controls during any of the trials.

To determine the viability of fire blight bacteria in honeycomb, commercial honey encased in a wooden frame with the comb in place was obtained. On October 3, 1936, minute droplets of fresh, sticky exudate obtained from blighted Bartlett pear shoots, were introduced into the honey cells, care being taken to see that each droplet was immersed in the honey. Likewise, small, 3- to 5-mm pieces of freshly blighted leaf petioles were immersed in other honey cells on the same date in order to determine any difference in reaction between the bacteria present in exposed exudate and those within blighted tissues.

EXPERIMENTAL RESULTS

Table 1 presents the results of these tests. The following additional data pertain to the materials and experimental conditions shown in table 1.

With reference to the four lots of exudate kept at the temperature recorded at 16° C., temperatures were read and recorded three times each week day—a procedure followed for all the different recorded temperatures shown in table 1. The readings for the 16° temperature showed but slight variation, the lowest being 15° and the highest 18°, the average or mean temperature being 16° and with only occasional variation from that figure. The material was kept in an incubator, especially useful for controlled low temperatures.

The different relative humidities were checked by means of hydrometer readings shortly after the last tests were made for viability and infectivity. These readings were interpolated between those presented in Stevens' table (*18*) in which various specific gravities of sulphuric acid-water mixtures are recorded in terms of relative humidities. The results of these comparative readings of the initial relative humidities and those obtained more than a year later are presented in table 2.

The temperature of 25° C. recorded in table 1 is only roughly accurate. The incubator utilized for this temperature held a fairly constant temperature when the laboratory temperature did not exceed 25°, but gave no good control at high laboratory temperatures. Daily readings throughout the period of investigation show that during the summer and early fall the readings fluctuated from 25° to 32°, although throughout the remainder of the year the temperature of 25° was fairly well maintained.

The 30° C. temperature shown in table 1 was held fairly constant in an incubator throughout the period of testing, with only slight fluctuations, which were usually of short duration. The fluctuations varied mainly from 28° to 32°, with one reading recorded at 36°.

The 35° C. storage temperature shown in table 1 was fairly well maintained in an incubator. The fluctuations varied from 32.5° to 40°, these being extremes. Most of the recorded fluctuations were between 34° and 36°.

TABLE 1.—*Viability and infectivity of Erwinia amylovora kept at various relative humidities and temperatures in the form of exudate, within diseased tissues, and as pure cultures*

Type of material	Approximate relative humidity	Temperature	Days viable and infectious [1]	Nonviable at any time tested (dates of tests)
	Percent	° *C.*	*Number*	
Exudate	0	16	[2] 320	
	9.5	16	[3] 571	
	21.0	16	[3] 571	
	45.0	16	[4] 400	
	0	25	[3] 412	
	9.5	25	[3] 412	
	21.0	25	[3] 421	
	45.0	25	[5]	
	0	30	[3] 408	
	9.5	30	[3] 674	
	21.0	30	[3] 408	
	45.0	30	----------	Jan. 22, Feb. 11, May 4, July 29, July 31, Aug. 7 and 13, 1936.
	0	35	[2] 320	
	9.5	35	[3] 426	
	21.0	35	[6] 320	
	45.0	35	----------	Jan. 14, Feb. 11, May 4, Aug. 18 and 26, 1936; Jan. 16, 1937.
	0	[7]	[3] 362	
	45.0	[7]	[3] 244	
	0	40	[8] 400	
	9.5	40	[8]	
	21.0	40	[9] 321	
	45.0	40	----------	Dec. 18, 1935; Feb. 11, May 5, Aug. 21 and 27, 1936.
	0	[10]	[11] 187	
	[12]	[10]	----------	Dec. 27, 1935; Jan. 14, May 25, 1936.
Diseased tissue	0	[13]	[3] 351	
	0	[14]	[15] 268	
Exudate	50.0	[14]	[3] 276	
	75.0	[14]	[10] 55	
	90.0	[14]	[17] 7	
Agar culture	0	[13]	----------	Jan. 15, Feb. 12, 1936.
Exudate	0	[13]	[18] 610	
Exudate in honey	[12]	[14]	[19] 22	
Diseased tissue in honeycomb.	[12]	[14]	[20] 121	

[1] This consists of the length of time in which viable and infectious bacteria were found in the different materials kept under designated humidity and temperature conditions, figured from the time that the material was first placed at the allotted humidity and temperature to the last date utilized for testing.
[2] No material remained for testing after the number of days indicated.
[3] No tests were made beyond the number of days indicated.
[4] Material not tested after 400 days.
[5] The material included in this test was accidentally destroyed early in the work.
[6] Tests made at the end of 426 and 444 days showed no viability.
[7] Outdoors (weather housing).
[8] Tests made at the end of 428 and 431 days showed no viability.
[9] Tests made at the end of 341 days and all later ones showed no viability.
[10] Outdoors (apple tree).
[11] Tests made at the end of 237 days and beyond showed no viability.
[12] Fluctuating.
[13] Laboratory.
[14] Outdoors.
[15] Material was inadvertently destroyed shortly after the last date of testing.
[16] Tests made at the end of 124 days and beyond showed no viability.
[17] Tests made at the end of 22 days and beyond showed no viability. The exudate at this constantly high relative humidity became coated with saprophytic molds within a few days time.
[18] Material kept over concentrated acid from June 12, 1934, to Feb. 11, 1936, when it was exhausted.
[19] Tests made at the end of 92 days and beyond showed no viability.
[20] Tests made at the end of 256 days showed no viability.

The 40° C. recorded in table 1 was also held fairly constant in an incubator, the usual fluctuations varying from 39° to 41°. Extreme fluctuations occurred on 4 different days, on 3 days when the temperature reached 44° and on another when it dropped to 28° (but restored in a few hours to the desired temperature).

As noted in table 1, the number of days in which the various materials showed the presence of viable and infectious bacteria represent the elapsed time from the placement of the materials under the designated controlled conditions to the date of the last test. This does not represent the ultimate length of time that may be involved in the longevity of *Erwinia amylovora* under those conditions in which the bacteria were found to be viable in the last test made.

Although an effort was made to use approximately equal quantities of inoculum for each viability test of exudate, in practice this was found to be impossible because of the variation in size of the droplets of exudate and the presence of epidermal hairs of host tissues to which many of the droplets were attached.

The exudate kept under fluctuating laboratory temperatures and under approximately zero relative humidity represents a continuation of the tests of the same lot of material presented in a former article (*16*). At the end of 610 days, the last date of testing, the material was exhausted.

TABLE 2.—*Comparison between initial relative humidities and those present more than a year later at different temperatures*

Approximate initial relative humidity	Incubation temperature	Specific gravity a year later	Temperature at specific-gravity determinations	Corrections for temperature in specific gravity	Approximate relative humidity more than a year later
Percent	° *C.*		° *C.*		*Percent*
0	40	1.815	36	1.835	0
21.0	40	1.460	36	1.476	21.5
45.0	40	1.350	36	1.365	45.0
0	35	1.815	37	1.835	0
9.5	35	1.570	36	1.586	9.5
21.0	35	1.460	36	1.476	21.5
45.0	35	1.350	36	1.365	45.0
0	30	1.815	33	1.833	0
9.5	30	1.570	33	1.584	9.6
21.0	30	1.460	33	1.474	21.5
45.0	30	1.350	33	1.363	45.0
0	25	1.815	32	1.832	0
9.5	25	1.565	31	1.578	9.6
21.0	25	1.460	31	1.472	22.7
0	16	1.820	23	1.831	0
9.5	16	1.580	21	1.584	9.5
21.0	16	1.470	21	1.474	21.5
45.0	16	1.355	21	1.358	45.2

It is to be noted in table 2 that the relative humidities obtained more than a year after they had been made up show slight difference or none at all from the initial ones. This is to be expected since the amount of moisture present in the droplets of exudate, or even in the blocks of agar cultures or host tissues, must have exercised an insignificant effect on the relative humidity in any one bottle because of the comparatively large volume of concentrated sulphuric acid or mixtures of this acid and water present. Likewise, the use of rubber stoppers and the immediate sealing with hot paraffin after material was removed for any one test may be expected to prevent any marked influence of air humidities on those present in the bottles. The breaking of the paraffin seal, the removal of material, and the recorking and resealing were conducted as quickly as possible. At all tests these were performed in a small, dry, completely enclosed transfer chamber.

LONGEVITY OF BACTERIA IN FREE EXUDATE

Evaluating and interpreting the results shown in the tables, first with reference to the exudate kept under controlled temperatures and under various relative humidities, one notes that at 16° C. the exudates at the four different relative humidities still yielded viable and infectious bacteria more than a year after the initiation of the tests. This is in contrast with the exudates kept at the higher temperatures in which the bacteria were found to be dead in the first tests at a relative humidity of 45 percent, while at the lower relative humidities, from approximately 0 to 21 percent, the bacteria were viable at all the temperatures utilized, including that of 40° C. for long periods. Obviously, relatively high temperatures, unless accompanied by moderate or high relative humidities, do not seem to possess any marked germicidal action against the fire blight organism when in the form of natural exudate. In other words, so far as temperature is concerned, *Erwinia amylovora* can live for long periods as exudate under very diverse temperatures, but is relatively short-lived at moderate or high temperatures when the relative humidity is moderate or high. In the presence of fairly low temperatures, relative humidity does not appear to be such a limiting factor, at least not under uniform conditions of temperature and humidity.

The actual length of life of fire blight bacteria at an approximate relative humidity of 45 percent and at temperatures of 25° to 40° C. was not determined. They were nonviable at the first and at all the subsequent tests, but the first test at 40°, owing to shortage of material, was not conducted until 181 days after the material was first placed under the controlled conditions, and at 35° and 30° not until 208 and 216 days, respectively.

With reference to the exudate kept under fluctuating temperature conditions, it is to be noted in table 1 that there were 10 different types of treatment: (1) Exudate kept at approximately zero relative humidity under outdoor conditions, exposed to sunlight; (2) similar to the first but in the shade offered by Weather Bureau-type housing; (3, 4, 5) similar to the second but kept at relative humidities of 9.5, 21, and 45 percent respectively; (6) exudate kept outdoors under fluctuating conditions of relative humidity and temperature, that is, without any effort to control the relative humidity and temperature; (7) exudate kept at approximately zero relative humidity but under fluctuating laboratory temperatures; (8) exudate kept outdoors at approximately 50 percent relative humidity; (9) exudate kept outdoors at approximately 75 percent relative humidity; and (10) exudate kept outdoors at approximately 90 percent relative humidity. As noted previously, the last three were maintained in a Weather Bureau-type housing. Owing to the fact that it seemed desirable for comparative purposes to place the material in glass bottles comparable to those used for the other tests, an error was introduced in two of the tests which throws considerable doubt on their validity. It was found that the temperature within the bottles in the presence of direct sunlight was such that in one of them, that used for fluctuating humidity and temperature, the heat and probably moisture, was sufficient to cause the individual droplets to run together while in the other the droplets turned jet black and gradually seemed to have lost much of their weight. While the bacteria were found to be viable and infectious at the end of 187 days at approximately zero relative

humidity under such outdoor conditions, they were nonviable at the end of 237 days; and the bacteria kept under both uncontrolled temperature and humidity conditions showed no viability in the first and in the subsequent tests. However, when the exudate kept at approximately zero relative humidity was placed outdoors in a Weather Bureau housing, the exudate was found to contain viable and infectious bacteria up to the last date of testing, a period of 362 days, or practically a year. Likewise, it is particularly worth noting that while the bacteria kept outdoors in Weather Bureau-type housing at high relative humidities died in short periods of time, that kept at 50 percent relative humidity showed viable and infectious bacteria throughout the whole period of testing, 276 days, or over 9 months.

The tests for viability and infectivity of the bacteria present in exudate kept under fluctuating laboratory temperatures and at approximately zero relative humidity clearly indicate that under such conditions the bacteria remain alive and infectious for remarkably long periods. It is obvious that the 610 days, shown in table 1, in which the bacteria remained viable, is not a complete picture, for the material was exhausted at that time. There are no data to indicate the actual span of life of *Erwinia amylovora* under such conditions, though it may be surmised that it is probably considerably longer than that given.

LONGEVITY OF BACTERIA IN AGAR CULTURES

Contrasted with the long life of bacteria in the form of natural exudate under fluctuating laboratory conditions and at approximately zero relative humidity, it is of special interest to note the short life of bacteria when in the form of pure nutrient-agar cultures under the same conditions. The first test, made 205 days after the agar smears had been placed over the concentrated acid, showed that the bacteria were dead, and a duplication of the test yielded the same result. The actual life span, evidently very short, of *Erwinia amylovora* in the form of such pure cultures, was not determined. If one may judge by the behavior of agar slants of this species, the bacteria rarely remain alive more than a week or two under such conditions. This marked difference in longevity as agar cultures compared with natural exudate suggested a study of the morphology of the bacteria when grown as agar slant cultures or in beef broth and as found in exudate.

LONGEVITY OF BACTERIA IN BLIGHTED TISSUE AND IN HONEY

The longevity of fire blight bacteria when present within blighted host tissues and kept at approximately zero relative humidity, both under fluctuating laboratory temperatures and under fluctuating outdoor temperatures, is apparently comparable to the longevity of the bacteria when in the form of exudate. At the end of 351 days, they were still viable when kept under laboratory temperature conditions within diseased host tissues, and also at the end of 268 days, under outdoor temperature conditions. Unfortunately, the latter material was accidentally destroyed in the field shortly after this period. It is worth noting that some of the pieces of blighted host tissues, when mashed in sterile water and used for a series of poured dilution plates, yielded almost pure cultures of a nonpathogenic yellow organism, with very few or no colonies of the whitish fire blight bacteria appearing in a series of 10 or more plates. Likewise, when a

water suspension made from such pieces was injected into healthy Bartlett pear shoots, no infections were obtained. It was found, however, that if a sufficient number of pieces of host tissues were used for obtaining heavy bacterial suspensions, and injected directly into healthy pear shoots, there was no difficulty in proving the presence of viable and infectious fire blight bacteria, even though the nonpathogenic, yellow bacterium was present. This is in line with former investigations (*12*) in which it was found that measured mixtures of these two different species of bacteria still permitted infections to take place in artificial inoculations, provided the mixture was not permitted to stand longer than 4 hours prior to the inoculations. However, if such a mixture, made up in the proportion of twice as many fire blight bacteria as the yellow saprophytic bacteria, is permitted to stand for 8 hours in nutrient broth, the former is completely destroyed at the end of that time. Obviously the use of blighted host tissues for determining the presence of viable *Erwinia amylovora* must be used with these facts in mind. Likewise, it is clear that if one introduces nutrient media containing pure cultures of fire blight bacteria into beehives or into any other open receptacle subject to contamination by common saprophytes, the fire blight parasite will be in competition with other organisms, many of which are far more rapid growers and can exercise deleterious effects on the parasite. The experiments on supposedly freshly infected tissues subject to invasion by secondary or saprophytic micro-organisms, suggest that unless a clear distinction is made between the capacity of this organism to live under conditions not subject to the activity of other micro-organisms and its capacity to live in an environment that is subject to the growth of other micro-organisms, any investigation seeking to determine the behavior of this parasite will yield conflicting or questionable results.

It is especially worth noting that the bacteria in the form of minute droplets of exudate are capable of living at least 22 days immersed in honey under such outdoor conditions as afforded by Weather Bureau housing. Under the same conditions, bits of diseased host tissues were found to yield live bacteria up to 121 days; at the end of 256 days they were no longer viable.

MORPHOLOGICAL STUDIES OF SLIMY ENVELOPES AS FOUND IN ARTIFICIAL CULTURES AND IN NATURAL EXUDATE

As indicated in a previous publication (*15*), there is a striking difference in the viscidity of this organism as it is found under pure-culture conditions compared with natural exudate. As fresh exudate issuing from infected tissues, it is so viscid that when touched with a needle it strings out in long, thin threads, clearly suggestive of a sticky or gummy slime gluing the bacteria to each other; grown in pure cultures in beef broth or nutrient agar, or in any other standard culture medium, it rarely shows such striking viscidity. This in turn signifies either that the enveloping membranes, slime, or capsules produced under one set of conditions are quite different, physiologically and morphologically, from those produced under the other set of conditions, or that the use of artificial media suffused with water dissolves or changes this slimy matter.

To gain more definite knowledge on this subject, the bacteria as obtained under the two different conditions were subjected to various

stains particularly applicable for showing slimy envelopes, and also studied under the microscope in a living condition, without the use of stains.

When a fresh, milky drop of exudate is touched with a sterile needle and the exudate drawn out into very fine threads approximately 10μ in diameter, these threads dry rapidly under ordinary laboratory conditions. They are readily cut into short lengths and can be placed on a glass microscope slide shielded by a cover glass for study of the bacteria in a living condition. Such studies make possible the observation of individual bacteria present in exudate without recourse to any medium such as water, alcohol, or stains that may produce artifacts.

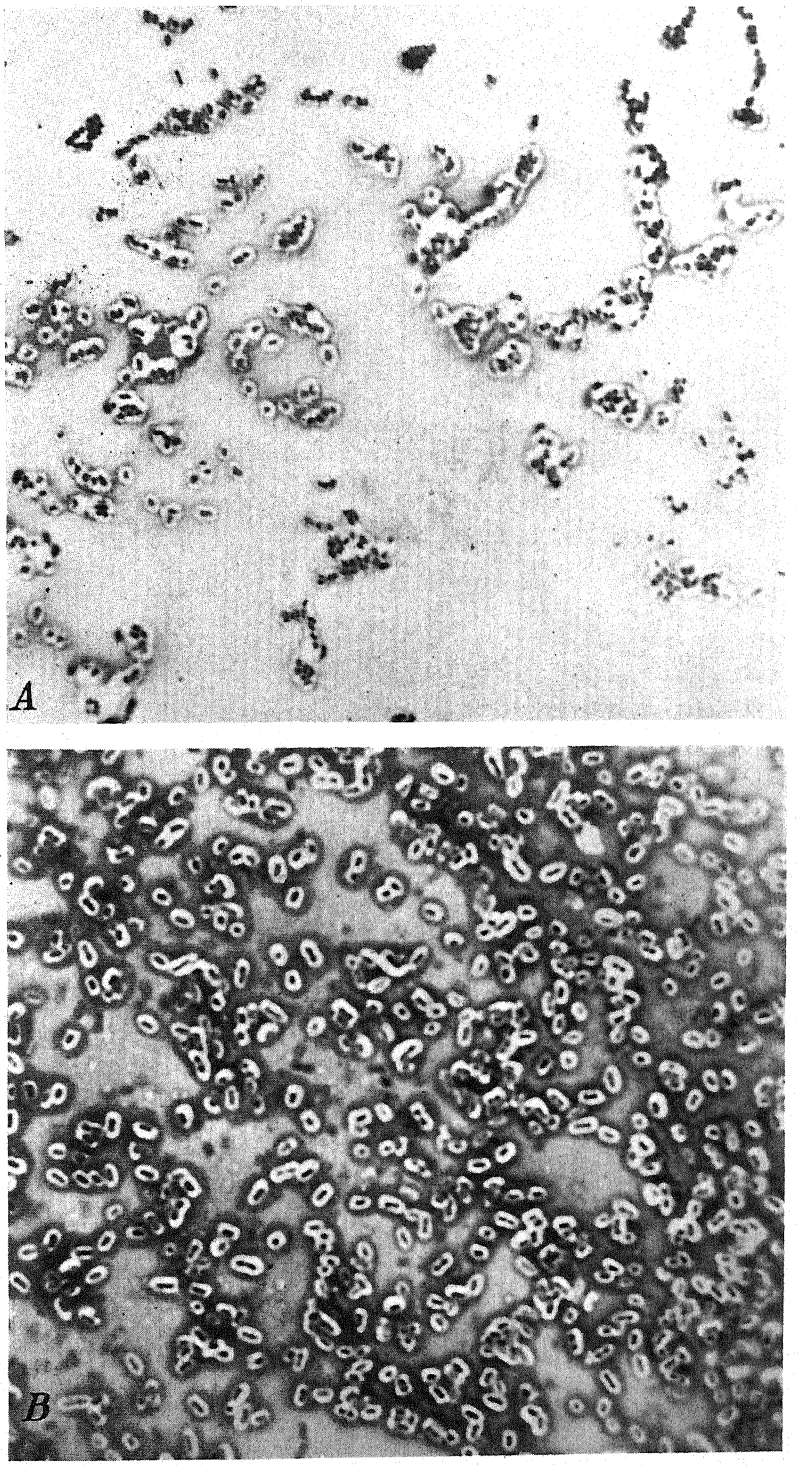
As observed under the microscope, the bacterial bodies within the thread are seen surrounded by a colorless substance taking the form of a halo which appears to be more glistening than the bodies, and which possesses a more or less definite outline. In general, the width of this substance as observed in these unstained, dry threads approximates that of the bodies and surrounds the bodies in much the same fashion as has been described for various encapsulated species of bacteria.

In contrast with these observations, when a drop of nutrient broth or syneresis liquid at the base of an agar slant, representing young, vigorously growing cultues of *Erwinia amylovora* are, without staining, examined in a hanging-drop slide, no such well-defined refractive substance can be seen around the bodies, although there are occasional suggestions of it. In such hanging drops, the bodies of the bacteria are usually much larger than in the dry bacterial threads.

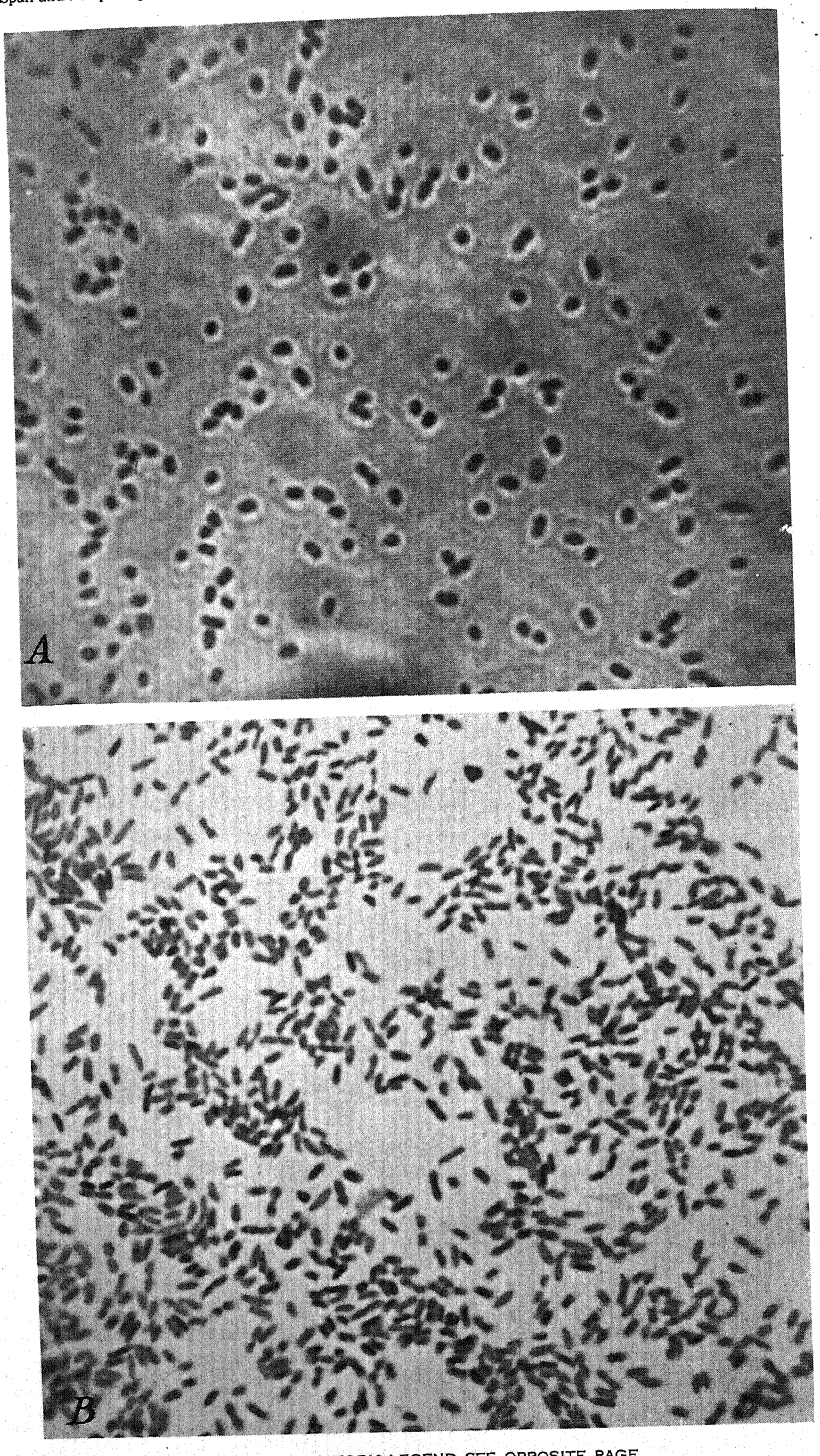
When a droplet of fresh exudate is placed in a drop of sterile water on a flamed slide, the bacteria will shortly cloud the water, indicating a dissolution or separation of the bacteria in the exudate. If this clouded water drop is then smeared over the slide, dried rapidly over a flame, stained for 20 to 30 seconds in a saturated solution of basic fuchsin dissolved in 95-percent alcohol, washed in running water, and examined under the microscope, there are very definite indications of a substance surrounding the bacterial bodies (pl. 1, *A*). If the bacteria are grouped or rather held together, a definite margined colorless substance, quite refractive to stains except at the margin, will be seen as a more or less continuous layer surrounding the group. If the bacteria are well isolated or separated from each other the individual bodies are often to be seen surrounded by the same type of envelope. However, this is not always the case, and the writer is not certain whether in such instances the enveloping layer has been lost somewhere in the process or that the technique does not work the same on all bacteria in a given smear. Since the bacteria representing the outer layers of exudate would be acted upon by the water for a greater length of time than the inner ones, there is of course the possibility that their enveloping layers would be subjected to greater

EXPLANATORY LEGEND FOR PLATE 1

Erwinia amylovora. *A*, Derived from fresh exudate, mounted in a drop of sterile water, dried rapidly over a flame, stained in a saturated solution of basic fuchsin dissolved in 95-percent alcohol, and washed in running water. Note presence of stainless material surrounding the bodies which is taken to be bacterial slime and which suggests capsulelike structures. × 1,725. *B*, Derived from exudate, kept outdoors at a relative humidity of 50 percent for 4 months, indicated the presence of viable and infectious material in inoculation tests, and was stained in the same way as that in *A*. Note presence of stainless material immediately around the bodies followed by an extreme outer layer of staining matter. These resemble encapsulated bacteria. × 1,820.



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change. It is well known that slimy envelopes are apt to be dissolved by water. Nevertheless, there is no difficulty whatever in finding many of the bacteria in such preparations with a clear-cut outer layer (pl. 1, *B*). Often the edge of this colorless layer is marked by a substance which is stained by the basic fuchsin, though not as deeply as the main bodies located within the colorless layer. This substance at the outer edge of the nonstaining layer is exceedingly elusive, often not at all observable or present merely as a trace (pl. 2, *A*). When this occurs, there is little or nothing to mark the outer edge of the colorless material unless some substance such as india ink or other extraneous matter is present which possesses an entirely different refractive index or stains readily. In the absence of any stained material at the margin of the stainless substance, the latter is practically unobservable.

CONTRAST IN STAINING REACTIONS

Contrasted with the highly refractive and often sharply delimited stainless material surrounding the bacterial bodies found in bacteria derived from exudate, no such well-defined nonstaining material is to be observed in bacteria derived from nutrient agar or broth cultures (pl. 2, *B*), although not infrequently there occur stainless areas around the bodies which suggest that they are not mere artifacts or light diffraction patterns but reduced or modified layers of comparable material found in exudate. Furthermore, old viable nutrient-broth cultures kept in a refrigerator will often show more or less of a viscidity when touched with a needle or loop, depending partly on the amount of agitation to which the cultures have been subjected. If such cultures are shaken vigorously, the viscidity especially of the upper liquid is readily lost, suggesting a dissolution or separation of the slime from the bodies, part of the slimy matter settling toward the bottom of the cultures. Upon decanting the upper liquid, the precipitate at the bottom still possesses some viscidity, varying apparently with the amount of agitation. Old, undisturbed cultures, in which most of the liquid has evaporated and which still possess viable bacteria, often show a degree of viscidity approaching that of bacterial ooze exuded from host tissues. There is, therefore, little doubt that *Erwinia amylovora* produces slimy matter in such cultures, although the slime is soon separated from most of the bodies, the separation depending upon the amount of water present and the degree of agitation.

In view of the fact that *Erwinia amylovora* has been described by various investigators as having no capsules, what are the enveloping layers, which are readily observable in bacteria derived from exudate—both in living material and in stained preparations?

The presence of these enveloping layers in living material suggests that they are not artifacts. Nevertheless, to make sure of this, fresh sticky exudate was placed on a glass slide in a mixture of india ink and water, the bacterial-ink preparation smeared over the slide with the edge of another slide as in making a blood smear, dried rapidly over a flame, stained with saturated basic fuchsin in 95-percent

EXPLANATORY LEGEND FOR PLATE 2

Erwinia amylovora. *A*, Derived from fresh exudate, mounted in 60-percent alcohol, dried, and otherwise handled like that shown in plate 1, *A*. Note reduced amount or absence of dark-staining matter at the outer margins of the colorless zones surrounding the bodies. Compare with plate 1, *B*. × 2,415. *B*, Derived from a 48-hour-old nutrient-agar-slant culture treated the same as that shown in plates 1 and 2, *A*, with which compare. Note very slight suggestion or none at all of slime or capsule formation. × 2,027.

alcohol, and washed in tap water. Preparations made in this manner almost invariably show a red-staining bacterial body surrounded by a well-defined colorless layer which is sharply delimited by the particles of india ink (pl. 3, *A*). The same structures appear in india-ink preparations without the use of heat or of staining, although the interior bodies being unstained or slightly so are not to be clearly distinguished, for photographic purposes, from the surrounding colorless layers. When nutrient agar cultures are treated with india-ink water mixtures there are also suggestions of colorless enveloping layers or capsules around the bodies but these layers are rarely ever as well defined or as large as in the bacteria derived from fresh exudate or from hard, dry exudate which contains living bacteria. Indeed, many of the bacteria derived from pure cultures in artificial media show no evidence of capsule or slime formation, although the evidence is apparently sufficient to indicate that *Erwinia amylovora* in pure cultures, especially in nutrient broth, produces some slime which is either readily shaken off or partially dissolved from the bodies of many of the bacteria. In this respect this species behaves somewhat differently from those human and animal bacterial pathogens which have been described as being encapsulated within the host and not in artificial cultures. The well-known behavior of *Bacillus anthracis* Koch is a good illustration of this (*6, p. 39*).

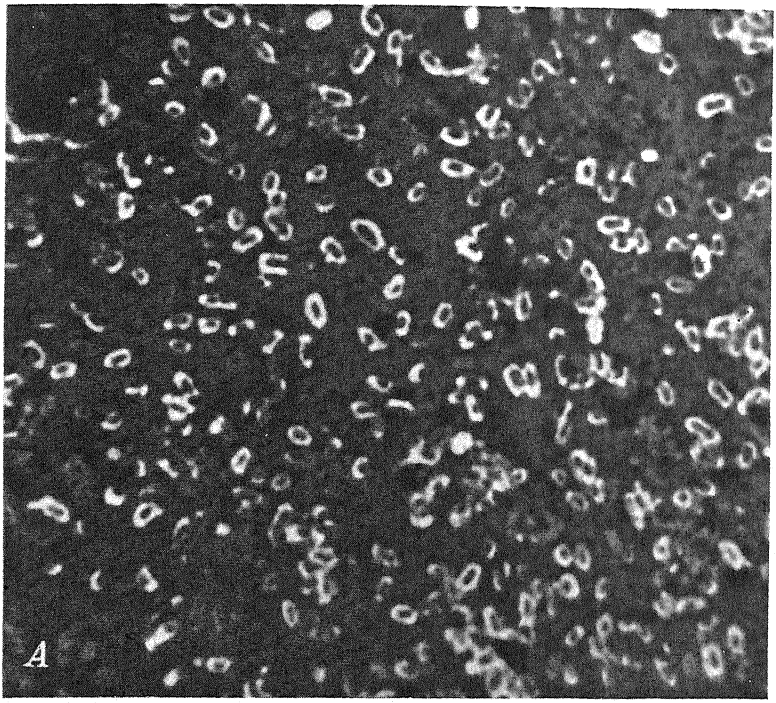
OTHER EVIDENCES OF ENVELOPES IN NATURAL EXUDATE

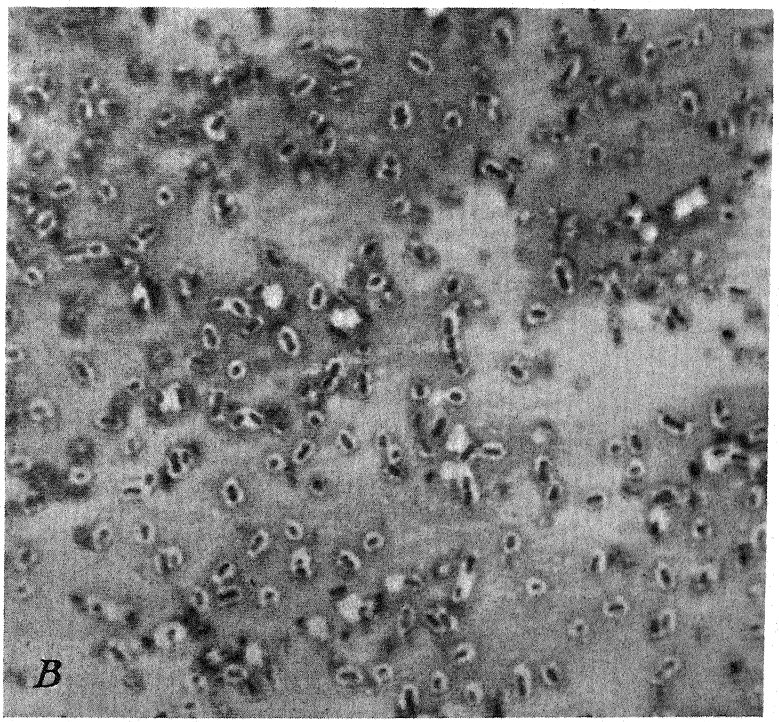
In addition to the above evidence for the presence of an enveloping layer around fire blight bacteria, especially those derived from exudate, there are two other observations which indicate the same thing. Since artificial cultures usually yield relatively few bacteria with definite indications of such layers, it seems fair to assume that the water contained in such cultures is probably responsible for their removal or partial disintegration. To test this theory, droplets of exudate were placed in sterile distilled water, using 2 droplets for approximately 1 cc, allowed to stand for 1 hour, the resulting cloudy suspensions smeared over glass slides, dried rapidly over a flame, stained with saturated basic fuchsin dissolved in 95-percent alcohol for 30 seconds, and washed with tap water. When such preparations are compared with similar exudate treated with a small drop of water for 2 or 3 minutes prior to the drying of the films, those which have stood 1 hour in water show enveloping layers that are wider but much less refractive and the outer edges are quite indefinite.

Still another line of evidence consists in the observation that bacterial bodies (protoplasts) are not infrequently entirely absent or located much to one side of stainless areas which assume the same general shapes and dimensions of the enveloping layers found in those bacteria with bodies intact and centrally located. There are several highly refractive, stainless areas without bodies or with bodies askew to be seen in plates 1, *A* and 3, *A*, and especially in plate 3, *B*. These

EXPLANATORY LEGEND FOR PLATE 3

Erwinia amylovora. *A*, In fresh, sticky exudate placed in a mixture of india ink and water, smeared over glass slide as in making a blood smear, dried rapidly over a flame, stained with a saturated solution of basic fuchsin dissolved in 95-percent alcohol, and washed with tap water. Note stainless layers around the bodies suggesting capsules or slime formation. × 2,500. *B*, From fresh exuate mounted in a drop of 60-percent alcohol, dried rapidly over a flame, and stained like that shown in plate 1, *A*. Note stained protoplasts centrally located in most instances, surrounded by stainless areas. Note also others in which the protoplasts are considerably to one side or outside of the stainless portions. × 1,725.





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areas apparently are not artifacts since a more or less regular series can be detected, varying from those in which the stained, centrally located bodies are evenly surrounded by the stainless halos, grading to those in which the bodies are decidedly to one side and extending to others in which the dark-staining bodies are entirely outside of the stainless areas. If stainless halos as a whole were mere artifacts consisting of a shrinking of the protoplasts and leaving an outer wall or layer of protoplasm separated from the main body by an unoccupied and hence stainless area, it is obvious that the shrinking occurred prior to the application of the stain; otherwise they would not appear. But, as there are numerous bodies appearing wholly outside of the stainless areas or to one side within the stainless portion, it seems clear that in these instances the bodies were probably dislocated or moved out of their natural position after the application of the stain. This is easily accomplished if the washing which follows the stain is too violent or prolonged, or if the cover glass is moved in the process of mounting, and various preparations have been obtained in which almost all the bodies were washed away or dislocated, leaving at times mere outlines of the enveloping layers.

If the stained protoplasts, centrally located or otherwise, represented shrunken bodies, they should appear more or less irregular in outline with disorganized parts evident, and staining irregularly. No such protoplasts have appeared in any of the preparations made for determining the presence or absence of enveloping layers by the method described above. As may be seen in any or all of the figures, protoplasts in sharp focus show definite and regular outlines and are stained homogeneously or with the well known bipolar staining reactions typical of normal bacteria. While the stainless zones around the protoplasts show considerable variation in size and shape, the protoplasts themselves are almost always regular. Furthermore, the size of these protoplasts plus the enveloping layers is considerably larger than those obtained for bacteria derived from the same exudate but stained with ordinary methods, such as Ziehl's carbol fuchsin, which do not reveal the outer layers. Often the bacteria which show these envelopes are fully twice as wide and as long as those which show no envelopes.

NATURE OF ENVELOPES

How to designate these outer slimy layers of *Erwinia amylovora* is a difficult question. Benecke (*3*) distinguishes two different types, the true encapsulated bacteria which produce gelatinous layers (Gallertbildung), and those which produce slimy layers (Schleimbildungen). The latter are distinguished by the ability of these layers to swell and to be soluble or deliquescent. However, he indicates that there are no clear distinctions between capsules and slimy layers (*3*, *p. 94*) and since he proposed no distinct term for the latter, the writer is inclined to call the slimy envelopes of *E. amylovora* "capsules" for want of a better term. Certainly, as observed in dry living threads, the bacterial protoplasts show these enveloping layers between them as structures just as fixed as in the recognized encapsulated bacteria. However this may be, it would appear far more important to recognize the fact that fire blight bacteria when derived from natural hosts, in the form of exudate, are encased in slime than to insist that these slimy secretions be called capsules.

The chemical nature of these slimy layers has not been fully investigated. Since the outer edges often take the basic fuchsin stain when the main part does not, there is a suggestion that the outer edge is in part proteinaceous. Likewise, since the greatest part takes no protein stain, it is probably mainly carbohydrate in composition. This appears to be in keeping with the findings of other investigators who have studied the composition of slimy bacterial envelopes. Ward (*20, p. 7*) in reviewing these studies calls attention to the following: In the Friedländer bacillus (*Klebsiella pneumoniae* Trevisan), Toenniessen has shown that the slimy envelope or capsule is made up of galactan and contains no protein. This was confirmed by Kramár who, in addition found that the capsule of *Bacillus anthracis* consisted of glycoprotein while that of *B. radicicola* Beijerinek was made up of a dextran. Likewise, Avery, Heidelberger, and Goebel found the capsular substance of pneumococcus type II to be a nitrogen-free polysaccharide and they found a similar carbohydrate in a strain of the Friedländer bacillus. Finally, in studying certain encapsulated strains of *B. coli* Migula, Smith found the capsule material to consist of 80 percent of hexose and a small amount of glycuronic acid. In short, all investigators here mentioned have found that slimy bacterial envelopes are composed either wholly of carboydrates or carbohydrates combined with proteins.

The conclusion is that the bodies of fire blight bacteria in the form of exudate derived from natural hosts are enveloped by slimy capsules and that this slime consists mostly of nonproteinaceous material. On the other hand, fire blight bacteria maintained as pure cultures in nutrient agar or broth rarely show such slime around the bodies. This is probably the reason why pure cultures show little or no viscidity when fresh exudate reveals it in striking form.

It is also concluded that the presence or absence of the slimy layers around the bodies probably exercises considerable influence on the longevity of the bacteria and on their ability to live under diverse conditions of temperature and relative humidity. At any rate, as shown in table 1, bacteria without slimy envelopes, those derived from nutrient agar, are very short-lived under the same conditions of temperature, humidity, and light that are conducive to exceptional longevity of bacteria that are encased in slimy envelopes derived from exudate.

DISCUSSION

The results obtained in subjecting bacterial exudate of *Erwinia amylovora* to a relatively wide range of temperatures and to various relative humidities indicate that neither of these factors taken by itself, within the ranges utilized, exercise an appreciable deleterious effect on the parasite, at least not for relatively long periods of time. To find a supposedly nonspore-forming species of bacteria capable of living at a temperature of 104° F. for at least 400 days, is in itself rather surprising since micro-organisms as a whole have been found in numerous investigations to be adversely affected by such high temperatures and rather resistant to temperatures much lower than the lowest utilized in the studies here reported.

These results are seemingly in disagreement with previous studies on thermal death-point determinations of *Erwinia amylovora* (*1, 14*) in which it was found that this parasite is quite sensitive to high temperatures, perishing in a few minutes when subjected to 43.7°,

45°–50°, 45°–46°, and 45.1°–48.3° C. for some isolates and 48.3°–49.5° for others, as found by different investigators. Actually, however, the writer is convinced that the disagreement may readily be explained by the difference in materials and methods rather than by any discrepancy in results. The five previous investigators all relied on a standardized technique based upon the use of such an artificial medium as peptone-beef broth or synthetic media. Clearly there must be considerable difference between the physiology and probably the morphology of a living thing growing in beef broth compared to the same organism which had made its growth within living host tissues and had been extruded from such tissues. The studies here reported on slime formation certainly suggest such differences.

The fact that at a relatively constant temperature of 40° C. (actually at least 4° higher at times), and at low relative humidities, the bacteria remain viable for at least 400 days suggests that when they are studied in the form of natural exudate the thermal death point will be found to be considerably higher. In any case the studies here reported not only indicate that *Erwinia amylovora* can withstand high temperatures for relatively long periods, but also suggest that the standard methods utilized for determining thermal relationships of bacteria in general are of doubtful value so far as indicating their reaction toward heat when they are grown under natural conditions.

While neither the temperatures nor relative humidities alone exercised a deleterious effect on *Erwinia amylovora* over relatively long periods, the data show as previously indicated that there is a very appreciable effect at higher temperatures when the relative humidities approach 45 percent. The interaction between moderate or high temperatures and high relative humidities apparently bring about a condition in which the bacteria are short-lived.

What is the possible significance of such behavior from the standpoint of available inoculum carried from one season to another? It is evident from these studies that too little attention has been given in the past to the possibility of this parasite being carried over in the form of exudate from one year to another. Almost all investigations on fire blight rest on the assumption that the inoculum is carried within host tissues blighted the year previous, in so-called hold-over blight. Why the bacteria should be considered as more likely to survive within host tissues than on the outside is not very clear. Aside from the influence of direct sunlight on exposed bacteria, the temperature of intercellular spaces, the main channels of bacterial invasion of host tissues, are probably proportional to those of the air. In strong light Curtis (*4*) found the internal temperature of apple leaves to be actually higher than the air temperature, while in diffuse light it was lower. In both instances the internal temperature was found to be directly proportional to that of the air. Similarly, Miller and Saunders (*9*) found the curves of surface temperature of leaves of various plants to follow those of air temperature pretty closely through a 24-hour period. With some plants the surface leaf temperatures are a little higher and in others somewhat lower than those of the air. So far as relative humidities of air compared with that of intercellular spaces are concerned, the writer has found no

definite data, although Shaw's studies (*17*) suggest that in succulent twigs of apple and pear the relative humidities are often much higher than that of the air. If this is true, then in accordance with the findings here presented the bacteria may be expected to be shorter-lived within the tissues than in extruded ooze since combinations of high relative humidities and moderate or high temperatures would occur more frequently within tissues than in the air of at least part of the fruit-growing areas found in this country. This, however, remains to be determined.

Considering the influence of humidity and temperature, the longevity of the bacteria as extruded ooze may be expected to be different in different sections of the country, depending at least in part on prevailing temperatures and relative humidities. Likewise, the longevity within tissues compared to that in exudate may be different.

The writer's studies of the longevity of *Erwinia amylovora* other than within host tissues have received encouragement in several interesting and promising lines of investigation, including the recent report of Ark and Thomas (*2*) on the persistence of this parasite in both fruit-flies and houseflies from pupa to adult, and also on the eggs of the latter. As such studies progress and include bacteria produced under natural conditions instead of artificial cultures, one may confidently expect to find these bacteria living for relatively long periods within a rather wide range of climatic conditions, in accordance with the studies here presented.

In addition to beehives it is obvious that any other nidus in and around orchards, where exudate may be carried by other insects or by rain and wind and where it may be shielded from direct rays of the sun, requires investigation before it can be certain that the bacteria are not living from season to season outside of host tissues.

SUMMARY

A study of the combined influence of temperature and relative humidity on fire blight bacteria in the form of natural exudate and as pure cultures revealed the following:

At a comparatively low, controlled temperature (16° C.) the bacteria as exudate remained viable and infectious at the four different relative humidities utilized (approximately 0, 9.5, 21.0, and 45 percent), throughout the entire period of testing, over a year.

At moderate and high, controlled temperatures (25°, 30°, 35°, and 40° C.) the bacteria in exudate also remained viable for long periods when the relative humidity was low. At 45 percent relative humidity they were dead when the first tests were made. It seems, therefore, that a combination of high temperature and moderate or high humidity is conductive to short life in this species of bacteria, while equally high temperatures with low humidities make for long life.

At uncontrolled or fluctuating temperatures, obtained by placing the exudate outdoors or on the laboratory shelf, the bacteria remained viable outdoors at 50 percent relative humidity for the entire period of testing, over 9 months, and at approximately zero relative humidity for almost 1 year, while at 75 and 90 percent relative humidities they died rapidly outdoors. At fluctuating laboratory temperatures the bacteria remained viable at approximately zero relative humidity for as long as the test was conducted, 610 days.

Contrasted with the long life of the bacteria in the form of exudate under fluctuating laboratory conditions, the bacteria obtained from artificial cultures are short-lived under the same conditions.

The longevity of fire blight bacteria within blighted host tissues has been found to be approximately equal to that of the bacteria in the form of exudate when the humidity is low.

Fire blight bacteria in the form of minute droplets of exudate were found to be viable and infectious at the end of 22 days when immersed in honey contained within comb kept outdoors in United States Weather Bureau-type housing. They were not viable at the end of 92 days under these conditions. As present within bits of diseased host tissues, also immersed in finished honey and otherwise under the same conditions as the exudate, the bacteria were found to be viable at the end of 121 days.

A morphological study of the bacteria derived from exudate indicates that they are enveloped in slimy capsules which are mainly non-proteinaceous. Similar studies of the bacteria derived from pure cultures also indicate that on artificial media such slimy layers are either dissolved or greatly reduced.

It is concluded that the presence or absence of slimy envelopes probably exercises considerable influence on the longevity of the bacteria under diverse conditions of temperature, humidity, and light; and that the bacteria encased in slimy layers, as they are in natural exudate, offer a fertile field of investigation relative to the epidemiology of fire blight, in the light of the studies here reported.

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FACTORS INFLUENCING THE DISCOVERY OF FOREST FIRES BY LOOKOUT OBSERVERS[1]

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INTRODUCTION

In forest-fire suppression activities there are four generally recognized elapsed-time steps between the start of a fire and the time suppression forces actually begin working on it. These are (1) discovery time, (2) report time, (3) get-away time of the initial attacking force, and (4) travel time of attacking force. To attain the promptness of attack desirable—termed "hour-control requirements"—in any given forest cover type, it is necessary to keep the sum of these four elapsed-time steps less than the total allotted in the hour-control set-up.[3]

The discovery time of fires, under the type of detection system employed throughout the national forests of California,[4] is subject to considerable variation, attributable to the varying physical conditions under which fires occur. This variation, unlike that in the other three elapsed-time steps, cannot be controlled by the protection organization. It is, however, desirable to measure the extent of the variations which occur and to associate them with their controlling physical factors in order that significant variations in discovery time may be estimated from the measurement of physical conditions under which fires occur.

The purpose of this paper is to present some measurements that have been made of the influence of certain factors on discovery time in the ponderosa pine (*Pinus ponderosa* Dougl.) type of northern California. While the quantitative experimental results obtained may not have direct application to other cover types or other forest regions, the trends established by the study may be of some value to all engaged in fire-control activities.

METHOD OF STUDY

In this study 200 actual test fires were set and allowed to burn from 10 to 30 minutes, depending upon their rate of spread, on the McCloud Flats of the Shasta National Forest during one fire season between July 1 and October 15.

Observations of the fires and quantitative measurements of the variables were obtained by two groups of observers. One, the ground crew, started and suppressed the fires and recorded weather and fuel data and the behavior of the fires. The perimeter of each fire was

[1] Received for publication September 20, 1937; issued April 1938.

[2] Maintained in cooperation with the University of California, at Berkeley, Calif.

[3] SHOW, S. B., and KOTOK, E. I. THE DETERMINATION OF HOUR CONTROL FOR ADEQUATE FIRE PROTECTION IN THE MAJOR COVER TYPES OF THE CALIFORNIA PINE REGION. U. S. Dept. Agr. Tech. Bull. 209, 47 pp., illus. 1930.

[4] BROWN, A. A. IMPROVING FOREST FIRE DETECTION IN CALIFORNIA. Jour. Forestry 33: 923-931. 1935.

plotted to scale at 2-minute intervals during the life of the fire. The second group consisted of a special observer on Black Fox lookout in the center of the area in which the fires were set and cooperating lookout observers on four other lookout points from each of which most of the area was directly visible. Each test fire set thus represented from two to five separate and independent discoveries. The special observer on Black Fox recorded the position of the sun at the start of each fire, a description of the background against which the smoke appeared, visibility conditions as indicated by visibility-meter ratings of natural objects in the landscape,[5] and discovery time. The cooperating lookouts recorded a description of backgrounds, general estimations of visibility conditions, and discovery time. Radio communication was maintained between the ground crew and Black Fox, and all lookout observers were notified in advance of the location and time of start of each fire. No attempt was made in this study to check the personal efficiency of the different observers.

The fires were confined mainly to pure ponderosa pine needles. This type was selected as being the most uniform in fuel character and the one in which the fires could be most easily suppressed. Analysis of the data pertains only to the pure pine type.

EFFECT OF FACTORS ON DISCOVERY TIME

Collection of field data was limited to fires that spread immediately upon ignition. The time in which an incipient fire in the form of a smoldering campfire or rotten log will actually start to spread and produce smoke is governed more by chance than by any measurable physical factors. Lookouts can normally be expected to discover only such fires as are spreading and have produced a definite column of smoke.

The important physical factors which influence the time from the start of spread of fires to their possible detection by lookouts are: (1) Atmospheric obscurity, (2) background against which the smoke is seen, (3) distance of the lookout from the fire, (4) relative position of the sun with respect to the observer's line of sight, and (5) relative behavior of the fire governing the rate at which smoke is produced and rises—this last being dependent upon the combined influences of wind velocity, moisture content, and nature and distribution of the fuel. The correlation of fire behavior with these governing factors is being undertaken as a separate project and has not been treated in the study of discovery time. In the collection of the field data, quantitative measurements were made of each of these factors for every discovery recorded, with the exception of background. This factor was described by its composition and appearance to each observer. The effect of topographic shadows upon discovery time was not considered, since they play a very minor role in the detection problem in northern California.

ATMOSPHERIC OBSCURITY

The degree of atmospheric obscurity was measured in terms of the clearness with which objects in the landscape could be seen. Because of suppression of many of the fires before they could be discovered by all the lookouts under poor atmospheric visibility conditions, data on

[5] BENNETT, M. G. A VISIBILITY METER. Jour. Sci. Instruments 8: 123-126, illus. 1931.

the influence of atmospheric obscurity are incomplete. Analysis of all the discoveries recorded, however, shows little quantitative relationship between the visibility of natural objects in the landscape and discovery time. This indicates that the concentration of particles in the atmosphere has a different effect upon the visibility of a smoke column from that upon the visibility of the landscape. Further work is required to find a more satisfactory method of measuring the degree of atmospheric obscurity as an influence on fire detection.

In order to simplify the analysis of the remaining factors, the range of atmospheric visibility encountered was divided into three broad classes based on visibility-meter ratings, and only those discoveries were used that fell within the upper class, representing excellent to moderate atmospheric transparency. This group constitutes approximately 86 percent of the observations made.

BACKGROUND

Green ponderosa pine timber, brush fields, mixtures of brush and timber in various proportions, and open flats covered with dry grass made up the backgrounds against which the test-fire smokes were observed. These were segregated into two classes to which differences in discovery time could be definitely attributed. The first and larger group includes the dark-colored backgrounds of brush, timber, and mixtures of the two. The data show no differences in discovery time caused by differences in background within this group.

The light-colored backgrounds of dry grass comprise only a very small portion of the experimental data and are insufficient in number to permit of detailed analysis. A summary of the data reveals quite definitely, however, that, under the same weather and fuel conditions, discovery time of smokes observed against backgrounds of dry grass is longer and much more erratic than that against the darker backgrounds of brush and timber.

A brief consideration of the factors controlling the intrinsic visibility of objects explains this. An object is visible to an observer because of contrasts in either brightness or color between the object and its background, or because of a combination of the two contrasts.[6] When either type of contrast is of a low degree, the other must be of a much higher degree, to cause the object to be visible. Smokes observed against brush and timber backgrounds are always brighter than their backgrounds and normally present a strong contrast in both brightness and color. Even when one type of contrast is low, the other is usually sufficiently strong to make the smoke visible. Smokes observed against the sky or dry-grass backgrounds, on the other hand, may be either somewhat brighter or less bright than their backgrounds or of the same brightness as their backgrounds, resulting in a low degree of contrast. This, combined with low color contrasts between smokes and light-colored backgrounds, causes such smokes to have a relatively low degree of visibility. Under these conditions such a fire, considerably prolonged, may not be discovered until the smoke column becomes large enough to appear against an adjacent darker background or to become visible through its increased size and

[6] NUTTING, P. G. THE VISIBILITY OF RADIATION. A RECALCULATION OF KÖNIG'S DATA. U. S. Bur. Standards Bull. 7: 235–238. 1911.
——— REPORT OF STANDARDS COMMITTEE ON VISUAL SENSITOMETRY. Jour. Optical Soc. Amer. 4: 55–79, illus. 1920.

changing brightness with increase in volume and density. Analysis of the remaining factors is confined to smokes showing against dark backgrounds.

DISTANCE

It is impossible to isolate completely the factor of distance between fire and observer. In a perfectly transparent atmosphere, the only effect of distance is to control the visual angle subtended by the smoke column at the eye of the observer. Within the limits of the experimental data, this effect is negligible when expressed in minutes of discovery time. Since the atmosphere is never completely transparent, however, the degree of transparency has an important influence on the apparent effect of distance upon discovery time.

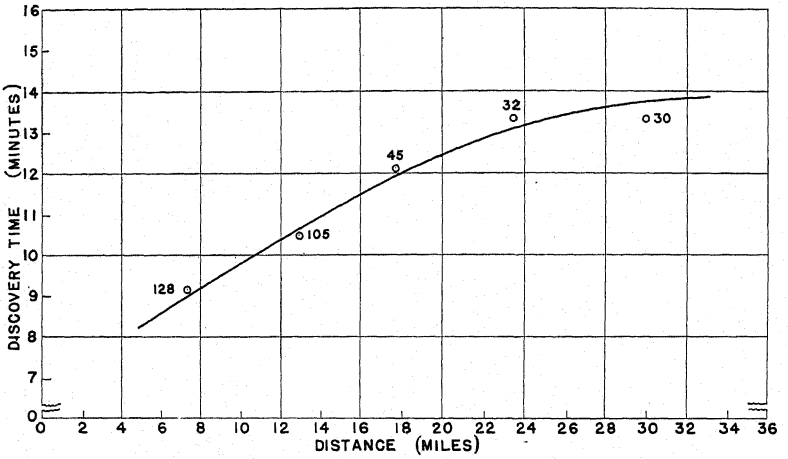


FIGURE 1.—Effect of distance upon the discovery time of pine fires under favorable atmospheric visibility. The figures on the curve indicate the number of observations taken.

The curve in figure 1, showing the increase in discovery time attributed to increases in distance, represents observations made under average good visibility rather than optimum conditions and therefore includes some effect of atmospheric obscurity. Within the limits of 15 miles, which is usually accepted as the maximum effective radius of vision of lookout observers, average discovery time may be considered as a straight-line function of distance under normal conditions. Distances greater than 20 miles, on the other hand, have a decreasing effect on discovery time because fires allowed to burn long enough to become visible at these distances normally begin producing smoke at a greatly accelerated rate. After this acceleration has begun, a large column of smoke is formed which becomes visible at relatively long distances within a very short time.

ANGLE BETWEEN SUN AND OBSERVER

Prior to analysis of the field data, a laboratory experiment was performed [7] to determine the effect of direction of illumination upon the visibility of a smoke column under controlled laboratory condi-

[7] BUCK, C. C., and FONS, W. L. THE EFFECT OF DIRECTION OF ILLUMINATION UPON THE VISIBILITY OF A SMOKE COLUMN. Jour. Agr. Research 51: 907–918, illus. 1935.

tions. From this experiment several conclusions were drawn, the most important of which was that the position of the light source (sun) with respect to the observer's line of sight, together with the concentration of particles in the smoke column, has a very definite effect upon the visibility of the smoke column. The results of this study materially aided in the analysis of this phase of the field data.

Following the trends indicated in the laboratory experiment, the effect of relative position of the sun upon discovery time was determined, as shown in figure 2.

This effect may be expected to vary somewhat with the degree of atmospheric obscurity. This in turn varies with position of the sun in the same manner as does the brightness of a smoke column, since the degree of variation in either is caused by the concentration of particles of suspended matter. The net variation in the visibility of the smoke column and its corresponding discovery time caused by variations in relative position of the sun is, therefore, determined by the relative concentrations of particles in the smoke column and in the atmosphere. Even under very adverse visibility conditions, however, in every observation made in the field with the sun behind the observer the discovery time of the fire was longer by at least a small margin than that of similar fires observed facing the sun.

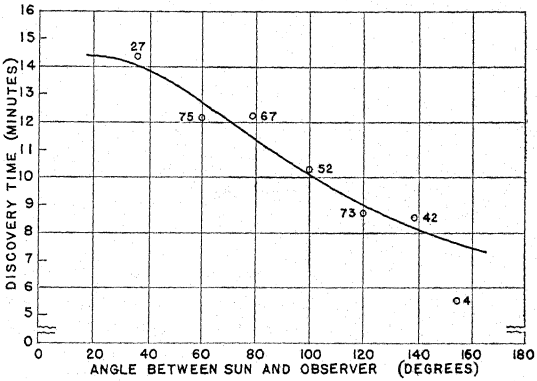


FIGURE 2.—Effect of relative position of the sun and observer's line of sight upon discovery time of pine fires under favorable atmospheric visibility. The figures on the curve indicate the number of observations taken. This is the angle with the smoke at the vertex and is the supplement of the angle measured at the observer between the sun and the smoke.

RATE OF SPREAD

Rate of spread is the principal element of fire behavior which influences the time required from the start of a fire for its smoke column to build up to the volume and density necessary to become visible to a lookout observer. Despite certain disadvantages, the unit of measure of this factor in the analysis was taken to be the average rate of spread for the first 8 minutes, since all fires were allowed to burn for at least this period. As a measure of rate of spread, average diameter, total perimeter, and area in square feet were all tried, but the last proved the most satisfactory in relation to discovery time. No usable relationship was found to exist between the size of a fire at any given moment and the size and density of its smoke column.

Figure 3 shows the average effect of the rate of spread of pine fires upon their discovery time under favorable atmospheric visibility. It will be noticed that discovery time decreases only slowly after rates of spread of approximately 40 square feet per minute are exceeded. This is due in part to the increased wind velocity responsible for the increased rate of spread, which undoubtedly has a marked influence

in keeping the smoke from rising above the timber crowns where it can be seen. Further, a rather definite minimum discovery time is determined by the failure of a fire to produce smoke for the first 1 or 2 minutes.

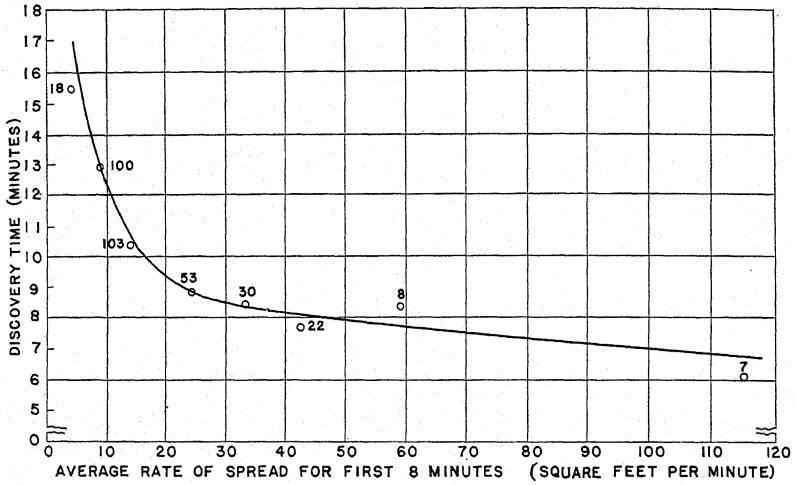


FIGURE 3.—Effect of average rate of spread upon the discovery time of pine fires under favorable atmospheric visibility. The figures on the curve indicate the number of observations taken.

COMBINED EFFECT OF RATE OF SPREAD, DISTANCE, AND POSITION OF THE SUN ON DISCOVERY TIME

To show graphically the combined influence of distance, position of the sun, and rate of spread upon discovery time, it is necessary either to make use of three-dimensional figures or to represent the influences of two of them with a single term. Analysis of the individual effects

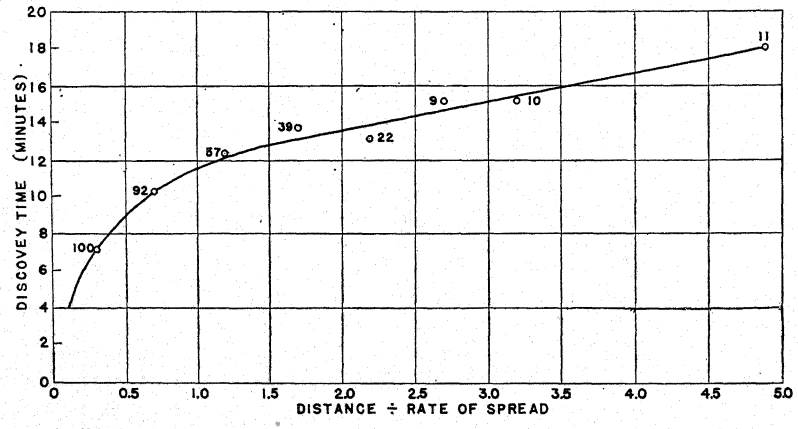


FIGURE 4.—Discovery time expressed as a function of the ratio between distance in miles and average rate of spread in square feet per minute for the first 8 minutes. The figures on the curve indicate the number of observations taken.

revealed that the latter method, involving the expression of discovery time as a function of the ratio between distance and rate of spread, could be used satisfactorily within the limits of the experimental data. This relationship is shown in figure 4 and indicates that under similar

conditions of position of the sun and atmospheric visibility, the discovery time of a fire 10 miles distant burning at an average rate of 25 square feet per minute for the first 8 minutes will be the same as that of a fire 20 miles distant burning at an average rate of 50 square feet per minute.

In figure 5, average rate of spread for the first 8 minutes and distance are represented on logarithmic scales in *A*. The ratio of distance to rate of spread is represented on a logarithmic scale by the diagonal lines in *A* which become the abscissae in *B*. Discovery time for different angles between sun and observer is plotted as the

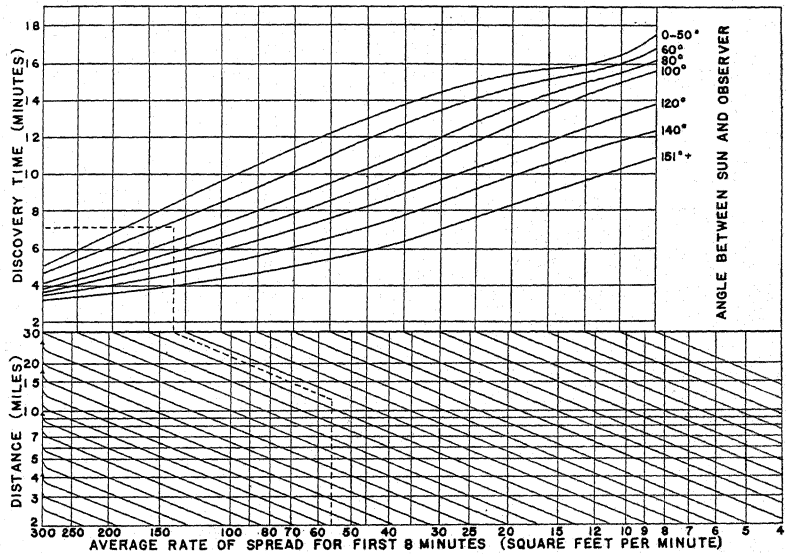


FIGURE 5.—Combined effects of average rate of spread, distance, and angle between sun and observer upon the discovery time of pine fires observed against brush and timber backgrounds under favorable atmospheric visibility. Example (shown by dotted line): Rate of spread 55 square feet per minute, distance 12 miles, angle between sun and observer 65°; discovery time 7.1 minutes.

ordinate in *B*. A total of 340 discoveries form the basis of this set of curves. Discovery time may be estimated from figure 5 in the manner illustrated by the broken line in the diagram.

Example: Rate of spread, 55 square feet per minute; distance, 12 miles; angle between sun and observer, 65°. Start at the value 55 on bottom scale and read vertically to distance 12 on lower left-hand scale; from this point follow upward to the left, parallel to the diagonal lines, to the heavy horizontal line, then vertically to the value 65° obtained by interpolating between the curves for 60° and 80°; discovery time is read on the upper left-hand scale opposite this point, 7.1 minutes.

A comparison of actual discovery times with estimated values from figure 5 indicates a relatively high degree of accuracy for observations during the middle of the day. Observations made at 7, 8, and 9 a. m. are more erratic. The heat from early morning fires is usually insufficient to puncture the inversion layer which characteristically persists in the experimental area from 15 to 30 feet above the ground at that time of day. The rising column of thin smoke from these early morning fires, upon striking the inversion layer, spreads out in

a horizontal plane and is soon dissipated. Discovery times of these fires may exceed the estimated values by as much as 10 minutes. Deviations of midday observations from the estimated values may nearly all be attributed to atmospheric obscurity. The experimental observations cover a range of distances from 3 to 33 miles and at the upper limits of this range even a slight haziness in the atmosphere undoubtedly has a detrimental effect upon discovery time.

SUMMARY

Better planning of protection organization developments and activities and better utilization of available man power are promoted by providing a more certain definition of elapsed-time allowances between start of fires and discovery than can be obtained from individual fire records. Solution of the discovery-time problem can contribute materially to this end. As a first step in this direction, the three discovery-time factors—distance, rate of spread, and position of the sun—were measured and their interrelation with respect to discovery time determined. The importance of these measurements, as shown in the accompanying charts, is that they form a basis for future work and at the same time may be used as trends indicating relative variations in discovery time which may be of value in present fire-control planning work. Their value will be enhanced materially as quantitative measurements are made of the effects of each of the remaining factors.

MECHANOGRAPHIC METHOD OF RECORDING INSECT CARDIAC ACTIVITY, WITH REFERENCE TO EFFECT OF NICOTINE ON ISOLATED HEART PREPARATIONS OF PERIPLANETA AMERICANA[1]

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INTRODUCTION

It is well known that many substances and experimental treatments may alter both the rate and the amplitude of heartbeat. This has been observed in a recent study of the effects of nicotine upon the contraction rate of isolated insect heart preparations (*6*)[3], in which a previously described method was used (*7*). That method yielded rate measurements only, and it was desirable to have a method that would measure both contraction rate and amplitude.

The present paper contains a preliminary description of a method by which rate and amplitude of the isolated insect heart preparation can be recorded photographically and measured. The results of the method are illustrated by tracings (mechanocardiograms) obtained from various segments of entire untreated and nicotinized isolated heart preparations of the American cockroach, *Periplaneta americana* (L.), and from completely isolated single segments.

METHOD

The mechanocardiograph method consists in transmitting the movement of the insect heart by means of a hair to a glass lever with an opaque long arm that moves in the path of a beam of light. The shadow of the moving arm is magnified and thrown across the slit of a camera containing a strip or roll of bromide paper. As the shadow moves, a tracing (mechanocardiogram) of the heartbeat is recorded upon the light-sensitive paper.

With the exception of the electrocardiographic camera, the set-up necessary for this method can be made from apparatus to be found in the ordinary physiological laboratory. The apparatus used includes the following parts:

The heart lever (fig. 1) consists of a capillary glass tube (*l*) of approximately 0.2 to 0.3 mm diameter drawn out to a smaller capillary tip (*ct*) at one end, so that the shadow of the capillary tip has a diameter of about 1.5 mm when magnified by a compound microscope with 16-mm objective and 10× ocular (tube length of 17.5 cm) and projected upon a screen 14 cm distant from the ocular. The lever is mounted with wax (*w*) upon and at right angles to a short but wider capillary

[1] Received for publication September 18, 1937; issued April 1937.

[2] The author is indebted to A. G. Galloway for his aid in connection with the modification of the electrocardiographic camera used in this work and to Saburo Katsura, of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, for the translation of Uramoto's article (*5*).

[3] Reference is made by number (italic) to Literature Cited, p. 275.

glass tube (*t*) of about 0.8 to 0.9 mm diameter, through which is extended an insect pin (*p*) of greater length and smaller diameter. The smaller end (*ct*) of the capillary lever is part of the long arm (*la*), and the larger end forms the short arm (*sa*) of the heart lever. If the lever is to be used horizontally and its shadow thrown across the vertical slit of an improvised kymographic camera (described below), the end of a straight human hair (*h*) is attached by means of wax to the short arm near the fulcrum, but if the lever is to be used vertically and its shadow thrown across the horizontal slit of an electrocardiographic camera, the end of the hair is attached (at *a*) to the wax (*w*) used to hold the lever to the supporting tube, so that the hair and lever are on opposite sides of the supporting tube (*t*). In either case a very small piece of lens paper (*lp*) is attached by means of a small bulb of wax (*b*) to the other end of the hair. The extremities of the insect pin (*p*, *p*) rest upon a support (*s*); a pair of curved forceps, held in a flat-jawed clamp, with the concavities of the jaws uppermost, which in turn is clamped to a heavy laboratory stand, can be used to support the insect pin. Special care is required to prevent vibrations of the table or building from being transmitted to the lever. The long arm of the lever should be rendered opaque, and in these experiments this was done by allowing Wright's blood stain to dry upon its surface. The exact balance of the lever necessary for the proper recording of heart movement can be obtained by trial.

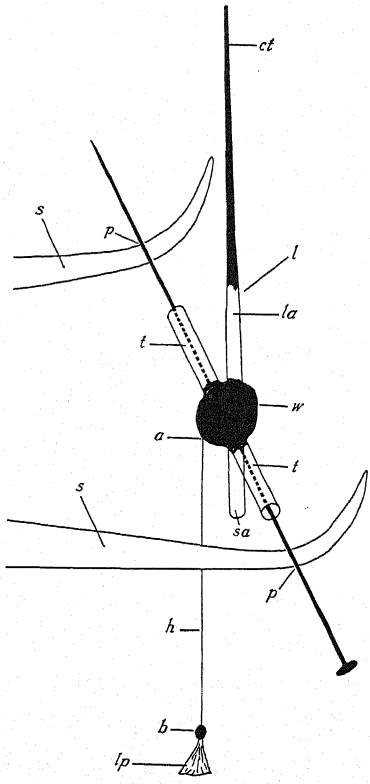


FIGURE 1.—The mechanocardiographic lever, showing its position when in use and held by forceps above the isolated heart preparation (not indicated): *a*, Point of attachment of hair to wax; *b*, wax, holding lens paper to hair; *ct*, opaque tip of heart lever; *h*, hair; *l*, heart lever; *la*, long arm of heart lever; *lp*, lens paper, used to make contact with heart; *p*, insect pin, at point of support; *s*, prong of lever support (forceps); *sa*, short arm of heart lever; *t*, supporting capillary tube of heart lever; *w*, wax, holding heart lever and hair to supporting capillary tube.

The relation of the heart lever to the other apparatus used in the setup is shown in figure 2. The beam of light (*b*) should be intense but with the heat removed by a water cell. The light is concentrated in the region of the tip (*ct*) of the long arm of the lever by a lens (*c*); the lower lens of a 10× ocular can be used for this. The source of light may be an improvised lamp containing a sufficiently strong light bulb, a lens, and a cooling water cell, or a manufactured research microscope lamp containing these parts.

The light, concentrated at the lever tip (*ct*), is passed into the 16-mm objective (*ob*) of a standard microscope tube removed from

its rack-and-pinion support, and clamped in a horizontal position to a heavy iron support; it passes out of the tube through a 10× ocular to the slit of the camera (*s*), which is placed at the desired distance from the ocular and across which the vertical lever shadow (*sl*) falls. These parts are so arranged that the beam of light is centered with respect to the optical system employed.

The camera can be improvised by placing a kymograph (a single-drum student kymograph has been used in some of these experiments) inside a box container that permits no light to enter when completely closed. A vertical slit, covered by a movable lip when not in use, is located in the wall of the container at such position that, when the lever's shadow is thrown across it, the section of the shadow that passes through the slit will fall upon a strip of bromide paper fastened about the kymograph drum and held only a few millimeters back of the slit. The kymograph can be started and stopped by means of a wire that runs from the outside through a small hole in the wall of the container to the starting mechanism. When the lever shadow is focused properly upon the closed slit, the kymograph is started, the slit opened, and the mechanocardiogram obtained; the slit is then closed immediately and the kymograph stopped. An improvised camera of this type is not satisfactory, for several reasons: The strip of bromide paper available for one continuous record is too short; focusing of the lever shadow is partly a matter of guesswork; and the entire camera must be transported to the dark room for loading and unloading. Although mechanocardiograms can be obtained with this improvised apparatus, it is much better to use a standard electrocardiographic camera that has been so modified as to run at required speeds. Most of the records obtained in the experiments reported here were made with a Hindle electrocardiographic camera (fig. 2, *e*) modified to yield paper speeds approximately of from 5 to 65 mm per second. In general, for work with insect hearts it is desirable to have available paper speeds of from 0.5 to 100 or more millimeters per second. Time markings can be obtained by using a watch time marker (*tm*) with a piece of black or opaque paper (*p*) attached to the tip of its recording arm (*a*) and arranged in such manner that the beam of light is interrupted at the required intervals (1 second, 5 seconds, etc.). A synchronous rotator with sets of rotating arms, such as is used with electrocardiographs, is preferable.

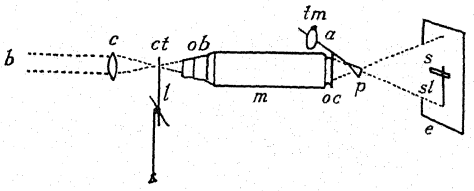


FIGURE 2.—Set-up of mechanocardiographic apparatus: *a*, Recording arm of time marker; *b*, beam of light; *c*, lens, used to condense light at heart-lever tip; *ct*, opaque tip of heart lever; *e*, slit-containing face of electrocardiographic camera; *l*, heart lever; *m*, microscope tube, with attached objective and ocular; *ob*, low-power objective on microscope tube; *oc*, 10× ocular in microscope tube; *p*, opaque paper on time-marker arm; *s*, slit in electrocardiographic camera; *sl*, shadow of opaque tip of heart lever; *tm*, watch-type of time marker.

The isolated heart preparation is dissected out in the manner described previously (*7*) and is pinned, ventral surface up, to the bottom of a suitable depression cut in a block of wax. The lever is attached to the heart by placing the lens paper (fig. 1, *lp*) on the dorsal dia-

phragm in the midregion just above the ventral surface of the cardiac wall. The tissues are bathed with physiological saline solution (1.25/11 Lévy's stock solution (*4*) has been used for *Periplaneta americana*) applied with a medicine dropper. In these preliminary experiments nicotine dissolved in the saline solution was applied in similar manner.

RESULTS

Some of the mechanocardiograms obtained by this method are illustrated in figures 3 to 7. Figure 3 shows the form of the record when the site of the lever attachment is the third abdominal segment of the isolated heart preparation. The time markings appear as vertical lines cutting across the entire width of the record, and denote intervals of 1 second. In this, as in all the records illustrated, the

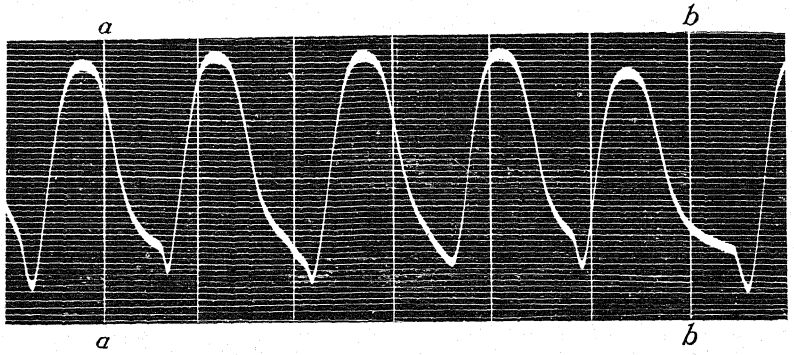


FIGURE 3.—A mechanocardiogram obtained from the third abdominal segment of an isolated heart preparation from *Periplaneta americana*. A rise of the curve represents a dorsally directed movement and a fall of the curve represents a ventrally directed movement of the ventral wall of the heart and the adjacent dorsal diaphragm. The vertical lines cutting entirely across the record are time markings denoting intervals of 1 second. The horizontal lines, made by the usual ruling behind the slit of the electrocardiographic camera, can be used to calibrate in terms of contraction amplitude. The record is to be read from left to right. Distance from line *aa* to line *bb* on original mechanocardiogram is 112 mm.

upward trend of the curve represents a downward (dorsally directed) movement of the dorsal diaphragm at its point of attachment to the ventral wall of the heart (i. e., at the site of attachment of lens paper, fig. 1, *lp*, to cardiac mechanism), while the downward trend of the curve represents an upward (ventrally directed) movement of the same region. Figure 4 shows a typical record obtained from the midabdominal region of the isolated heart preparation and analyzed into its component regions. Under the ordinary conditions of heartbeat and under the conditions of these experiments, the insect cardiac cycle in the midabdominal region of the heart consists of a systolic period (*sy*), represented in the mechanocardiogram by the marked sudden rise of the curve; a diastolic period (*dl*), represented by the marked sudden fall of the curve; and a diastatic period (*dt*), represented by the approximately horizontal region of the curve preceding the succeeding contraction. Often a sudden fall of the curve immediately precedes the systolic rise, appearing as a "presystolic notch." It can be seen also that the diastolic fall of the curve occurs in two fairly distinguishable stages, the first (*1dl*) including the earlier, sudden down stroke of relaxation, and the second (*2dl*) including the

later, less rapid downward trend, which finally becomes the diastatic region.

The mechanocardiogram can be obtained from a single isolated segment, separated from the rest of the isolated heart preparation by complete transverse sections made immediately in front of and immediately behind the required segment. Examples of such records are shown in figure 5. In general, the form of the curve is about the

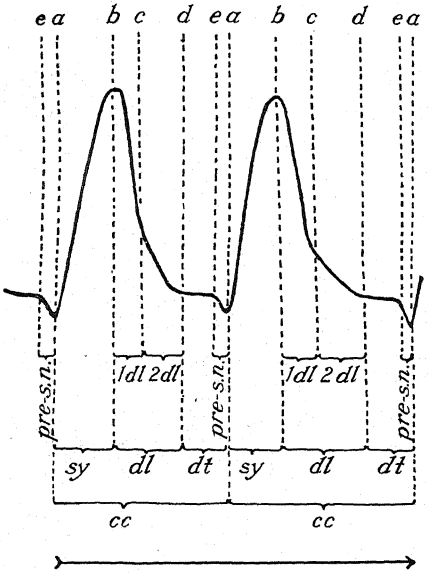


FIGURE 4.—A diagram representing two complete cardiac cycles, analyzed into their component parts as indicated by the vertical broken lines and by the lower braces. The curve is to be read from left to right, as indicated by the arrow: *cc*, Cardiac cycle; *sy*, systole; *dl*, diastole; *dt*, diastasis; *pre-s. n.*, presystolic notch; *1dl*, first part of diastole; *2dl*, second part of diastole; *a* . . . . *e*, lettering to distinguish vertical broken lines.

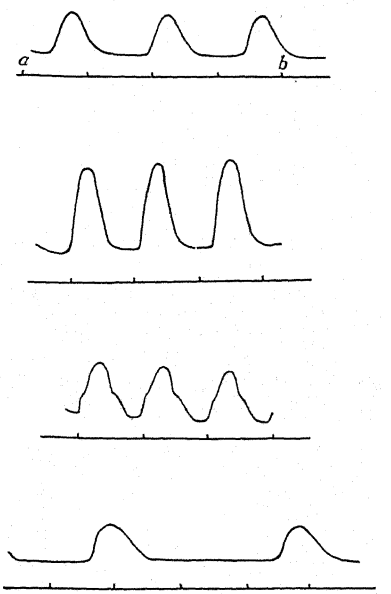


FIGURE 5.—Tracings of mechanocardiograms obtained from completely isolated single abdominal segments separated by transection from other segments of the isolated heart preparation. Time intervals of 1 second are indicated below each curve, which is to be read from left to right. Distance from point *a* to point *b* (top curve) on original mechanocardiogram is 66 mm.

same as that shown in figure 3, except that usually the presystolic notch is lacking.

Mechanocardiograms obtained from various segments of the isolated heart preparation are shown in figure 6. In this figure, 3*th* is obtained from the third thoracic segment, 1*abd* to 6*abd* from the first to sixth abdominal segments, and 7+8 *abd* from the seventh and eighth abdominal segments. Since these records were obtained with the same lever arrangement, the magnification is approximately the same for all. It can be seen that the amplitude tends to be greatest in the region of the fourth abdominal segment.

The effects produced in the mechanocardiogram by the application of nicotine to the isolated heart preparation are shown in figure 7. "Normal" represents the mechanocardiogram obtained from the nonnicotinized isolated heart preparation bathed with physiological saline solution. This record, like the others of this figure, was taken

with a lower camera speed than those of the preceding figures. The curves "Nicotine 1" to "Nicotine 3," inclusive, represent records made at successive time intervals after the application of nicotine to the heart preparation. The curves "Recovery 1" to "Recovery 4," inclusive, represent successive stages of recovery after the nicotinized preparation had been washed with fresh physiological saline solution. As the nicotine acts upon the cardiac mechanism, the mechanocardio-

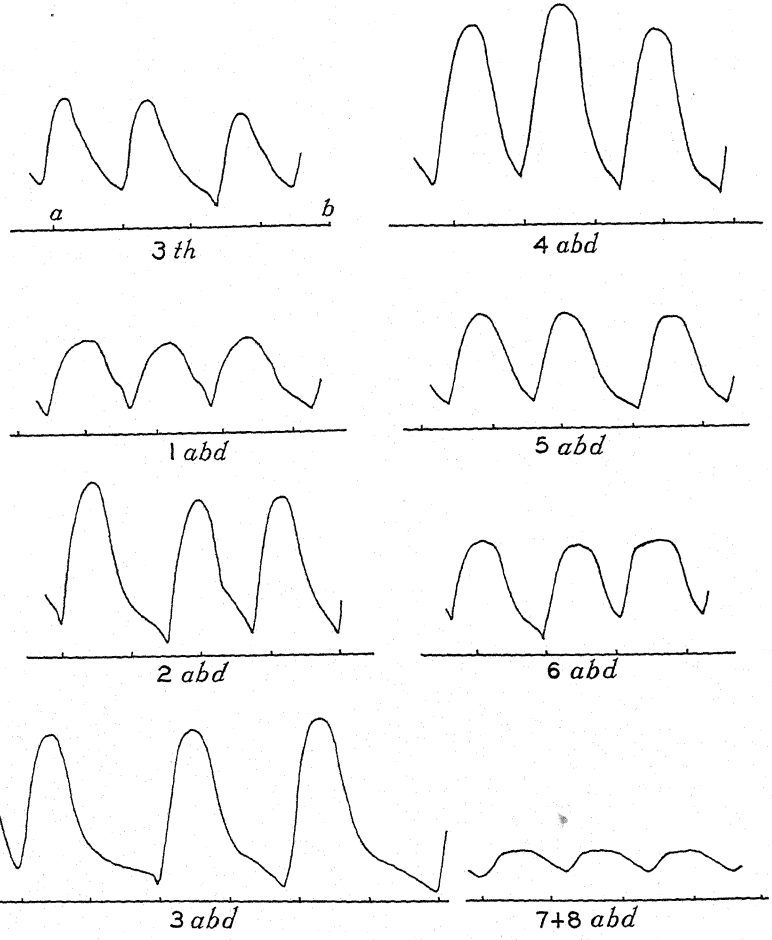


FIGURE 6.—Tracings of mechanocardiograms obtained from various segments of the isolated heart preparation, as indicated by the following labeling: *3th*, Third thoracic segment; 1*abd*, first abdominal segment; and so on through the sixth abdominal segment; 7+8 *abd*, seventh and eighth abdominal segments considered together. The curves are to be read from left to right. Time intervals of 1 second are indicated below each curve. Distance from point *a* to point *b* (*3th*) on original mechanocardiogram is 75.5 mm.

gram shows increasing irregularity of amplitude. Some irregularity of contraction height occurs, but a greater irregularity appears in the degree of diastolic fall of the curve; as the nicotine effect increases, the diastolic fall becomes less and less, until eventually the heart approaches systolic arrest. During recovery relaxation becomes more complete, marked irregularities of contraction height persist for

a shorter or longer time, and premature and partially summated contractions tend to persist for a relatively longer time; eventually, however, the form of the mechanocardiogram approaches that of the heart preparation preceding nicotinization (Recovery 4). With

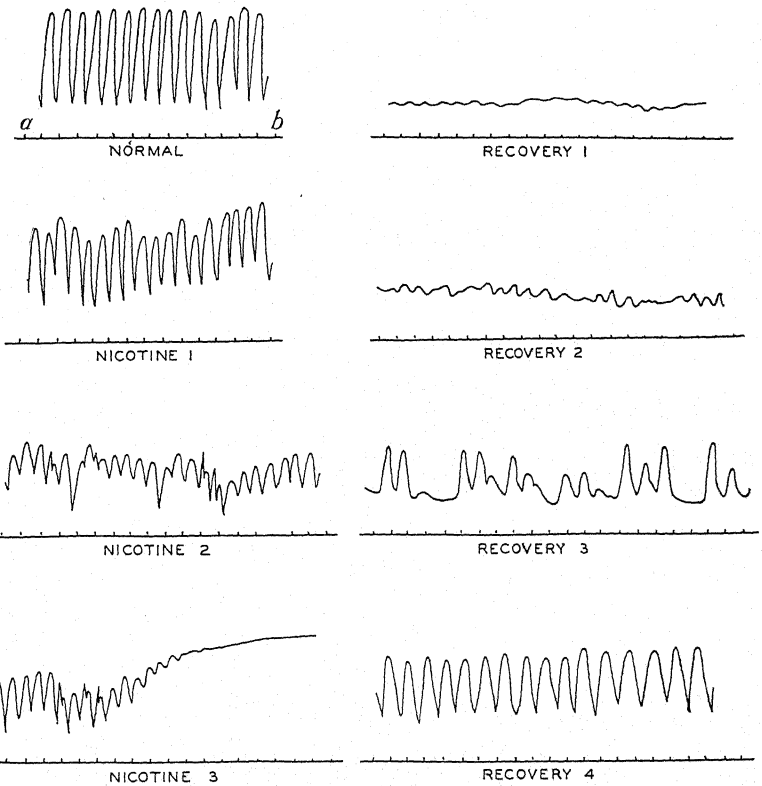


FIGURE 7.—Tracings of mechanocardiograms showing the effect of nicotinization of the isolated heart preparation. The curves, to be read from left to right, are from a single heart preparation. Normal tracing was made before nicotinization. Nicotine 1, 2, and 3 are tracings obtained at successive time intervals after application of nicotine. Recovery 1, 2, 3, and 4 are tracings made at successive time intervals after the heart preparation was washed with fresh saline solution. Distance from point *a* to point *b* (Normal) on the original mechanocardiogram is 74 mm. Time intervals of 1 second are indicated below each curve.

continued washing of the nicotinized tissue with fresh saline solution, the original form of the mechanocardiogram can be restored, provided the nicotinization has been neither too intense nor too prolonged.

DISCUSSION

The idea of utilizing a hair to transmit insect cardiac contractions to a small lever and photographing the lever movement has been applied by Kojantchikov (*2*) in a study of the heartbeat of the cockroach (*Periplaneta*) *Blatta orientalis* L. Kojantchikov's lever made contact with the heart by means of a piece of fat body tied to the free end of the hair, and, depending entirely upon the lever for magnification of heart movement, made a photographic tracing with a kymograph camera. The mechanocardiograms thus obtained consisted of only slight fluctuations of the curve, the highest magnification

being about three times. Uramoto (*5*) obtained smoke-drum (kymograph) tracings of the heartbeat of the silkworm larva by means of a light straw lever connected with the heart (isolated) by means of cellophane or resting upon the dorsal wall of the intact insect. Since, in making these records, magnification of heart movement was by means of lever only, the fluctuations in the kymograph tracings were small. In the method described here both magnification by lever and magnification by an optical projector system have been utilized, with the result that mechanocardiographic curves of sufficient size to permit analysis have been obtained. Most of the records referred to in this paper were made with magnifications of 400 to 600, but much greater magnifications can be obtained when desired. The factors determining the desired magnification are the width of the bromide paper, the intensity of the illumination, and the size and distinctness of the edge of the lever shadow. Although mechanocardiograms obtainable by this method are superior to those published by Kojantchikov (*2*) and Uramoto (*5*) and are more easily subjected to analysis, these investigators were the first, insofar as the author is aware, to obtain mechanocardiograms of the insect heartbeat.

From figure 6 it can be seen that the relative amplitude and the detailed form of the mechanocardiogram vary with the site of the lever attachment. Usually the largest relative amplitude is from the midabdominal region (fourth or fifth abdominal segment).

The significance of the presystolic notch is not entirely clear. Theoretically it should result from any presystolic force or forces that produce a ventrad movement of the ventral wall of the heart or of the adjacent region of the dorsal diaphragm. From a consideration of the anatomical relationships of the dorsal diaphragm to the cardiac tube in the American cockroach, it becomes apparent that the presystolic notch may be the result of alary muscle contraction, especially of those alary muscle fibers that lie approximately in the plane of the dorsal diaphragm. It is also obvious that the presystolic notch may result from a passive dilation of the cardiac tube by an increased pressure of intracardiac fluid resulting from a contraction wave occurring in another region of the heart than that producing the mechanocardiogram. Some evidence has been obtained that the presystolic notch can result from increased intracardiac pressure produced in the manner just suggested. No evidence has yet been obtained as to whether the presystolic notch may also be caused by alary muscle contraction. The correct interpretation of the presystolic notch must await further investigation.

In these experiments, as in those of Kojantchikov (*2*), it has been observed that as the rate of heartbeat increases the length of the diastatic period decreases until, with a rapid heartbeat, it may entirely disappear, in which case each systole begins at some point in the last portion of the diastolic period.

At a given moment the effect of nicotine upon amplitude may be relatively independent of its effect upon rate of contraction. In figure 7, Nicotine 1 and Nicotine 2, the mechanocardiograms were obtained at times during the onset of the nicotine effect when the rates of beat were not very different from that of the preparation before nicotinization; yet the contraction amplitude is markedly affected, especially with respect to relaxation. The cardiac mechanism tends to remain in a systolic condition and eventually (Nico-

tine 3) comes to systolic arrest. This observation is in agreement with the conclusion of Burridge (*1*) and Langley (*3*) that nicotine tends to decrease the ability of striated muscle to recover from the contraction state.

The method described here is applicable not only to the cockroach heart but also to the hearts of many other insects, provided the size and form of lever and its hair attachment to the heart are adapted to the particular species of insect employed. The method can also be used to record movements of various parts of the alimentary tract of the dissected preparation and to study the effects of pharmacological and toxicological substances upon various regions of the alimentary tract and other mechanically active portions of the insect body.

SUMMARY AND CONCLUSIONS

With the photographic method described in this paper, records (mechanocardiograms) of the insect heartbeat can be obtained of sufficient size and clarity to serve for analysis of the cardiac mechanism and the effects of various factors acting upon it, including the effects of various pharmacological and toxicological substances; it can be similarly used with respect to the alimentary tract or other mechanically active portion of the insect body.

Both rate and amplitude of insect heartbeat in the isolated preparation can be simultaneously and quantitatively measured by this method.

From the preliminary experiments reported here, the following conclusions have been reached with regard to the isolated heart preparation of the cockroach *Periplaneta americana* (L.):

The detailed form of the mechanocardiogram varies with the site of attachment of the lever to the heart, but in general, with a low rate of beat, indicates a cardiac cycle consisting of systole, diastole, and diastasis.

As the rate of beat increases, the diastatic (rest) period tends to become shorter and, with a sufficiently high contraction rate, may disappear.

Usually the systolic rise of the mechanocardiographic curve is preceded by a marked sudden fall, for which the name "presystolic notch" is suggested.

The significance of the presystolic notch is not completely apparent, but evidence has been obtained that it can result from increased intracardiac pressure.

Nicotine can markedly affect amplitude of beat with relatively little simultaneous effect upon contraction rate.

Nicotine appears to decrease the ability of the heart to relax, with the result that it may be brought to systolic arrest.

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THE TOXICITY OF PHENOTHIAZINE AND SOME OF ITS OXIDATION PRODUCTS, IN EXPERIMENTS WITH CARASSIUS AURATUS [1]

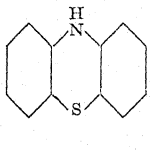
By W. A. GERSDORFF, *associate chemist*, and H. V. CLABORN, formerly *junior chemist, Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

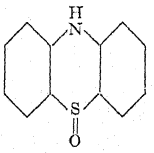
Recently it was reported [2] that, in the course of pharmacological experiments on rats with a newly proposed insecticide, phenothiazine (*2, 8*),[3] evidence had been found in the feces of the rats of a colored derivative of this substance that possessed strong bactericidal properties. This suggested the possibility of the existence of toxic oxidation products of phenothiazine. Unpublished results of tests for insecticidal action, however, with two such products, phenothiazone and phenothiazine sulphoxide, made by E. H. Siegler, of the Division of Fruit Insect Investigations, against larvae of the codling moth, and by D. E. Fink, of the Division of Control Investigations, against mosquito larvae, have indicated little, if any, toxicity of these compounds to these insects in comparison with that of phenothiazine.

A study of this subject was undertaken by the authors with a method in which the goldfish (*Carassius auratus*) is used as a test animal. Because the goldfish is very susceptible to certain types of poisons, relatively low concentrations are permitted, thus increasing the chances of the tests being made within the range of solubility.

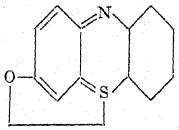
The substances studied were phenothiazine, phenothiazine sulphoxide, phenothiazone, and thionol. For comparative purposes a series of tests was also made with rotenone. The relationships of phenothiazine and its derivatives are shown by their structural formulas.



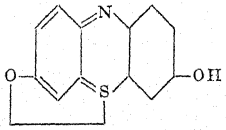
Phenothiazine



Phenothiazine sulphoxide



Phenothiazone



Thionol

Phenothiazine was prepared by the method described by Smith and his coworkers (*8*). One mole of diphenylamine and two moles of sulphur were fused at 180° C. with the use of iodine as a catalyst. The reaction product recrystallized from toluene gave the pure phenothiazine, a light yellow crystalline compound melting at 180° C.

Phenothiazine sulphoxide was prepared by the oxidation of phenothiazine with hydrogen peroxide as described by Pummerer and Gassner (*7*). It is a slightly yellow crystalline compound which melts at 250° C.

[1] Received for publication Nov. 13, 1937; issued April 1938.

[2] By Floyd De Eds, J. O. Thomas, and C. W. Eddy, of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, and A. B. Stockton, of Stanford University Medical School, at the meeting of the American Society for Pharmacology and Experimental Therapeutics at Washington, D. C., on March 26, 1936.

[3] Reference is made by number (italic) to Literature Cited, p. 282.

Phenothiazone is a purplish red crystalline compound melting at 162° C. It was prepared by the oxidation of phenothiazine with anhydrous ferric chloride by the method described by Pummerer and Gassner (*7*).

Thionol was prepared by treating thionin with sulphuric acid according to a method described by Bernthsen (*1*), although it was found necessary to decrease the sulphuric acid to 65 percent. The compound is a greenish-black crystalline powder having a metallic luster. It does not melt sharply; but, since it is soluble in both concentrated hydrochloric acid and ammonium hydroxide and gives all the color tests reported by Bernthsen, there is no doubt as to its identity.

EXPERIMENTAL PROCEDURE

The method of making the toxicological tests was essentially the same as that described in a previous paper (*3*). Goldfishes of a single lot were used, and a constant temperature of 27° ±0.2° C. was maintained. The fishes weighed approximately 5 to 7 g.

Except in the case of phenothiazone, acetone was used as a dispersive agent, stock solutions of the substances being prepared with it, from which the proper aliquots were taken for the tests. In the making of the test solutions, a higher concentration of acetone than 1 cc per liter of water, which is a concentration nontoxic to goldfishes weighing 2 g or more, was avoided. Phenothiazine and phenothiazine sulphoxide are so insoluble in water and, possibly only on that account, so slightly toxic that in the high concentrations needed they precipitated quickly. Thionol is but slightly soluble in acetone and alcohol (which could have been used in place of acetone) as well as in water, and the acetone used in the tests was little more than a wetting agent. As phenothiazone is soluble in water at the concentrations used, this solvent was used in preparing stock solutions. Acetone was used, as is customary, in the preparation of the test solutions of rotenone; the aqueous solution, however, was apparently complete at the highest concentration used.

RESULTS

The toxicological data are given in table 1. It is at once apparent that phenothiazone, the only one of the sulphur compounds readily soluble in water, has a comparatively high toxicity to goldfish of this size. The toxicity of this substance as compared with that of rotenone is seen from the survival-time curves of figure 1 and the velocity-of-fatality curves of figure 2. Curves are also drawn for phenothiazine, but it must be emphasized that they are only an indication of toxicity under the conditions of suspension.

The frequency distributions of the observed survival times for both rotenone and phenothiazone do not have the pronounced skewness characteristic of phenolic compounds previously studied (*5*, *6*), which led to the use of geometric rather than arithmetic means. Those of rotenone, however, are much more skewed than usual, seemingly because of the much greater resistance of these larger goldfish to this compound. In view of this, and also to make possible ready comparison with recent work, geometric means were again used in drawing the graphs and in expressing relative toxicity.

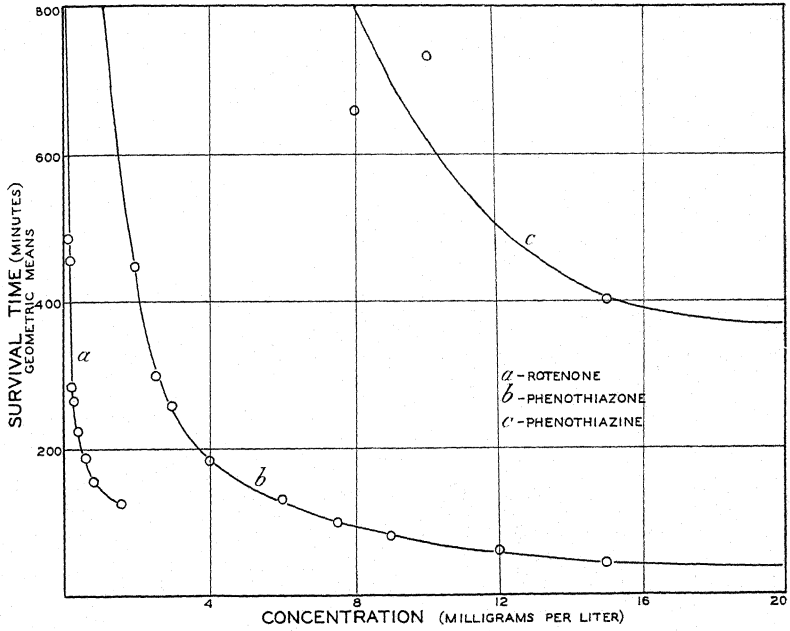


FIGURE 1.—Survival-time curves for phenothiazone, rotenone, and phenothiazine.

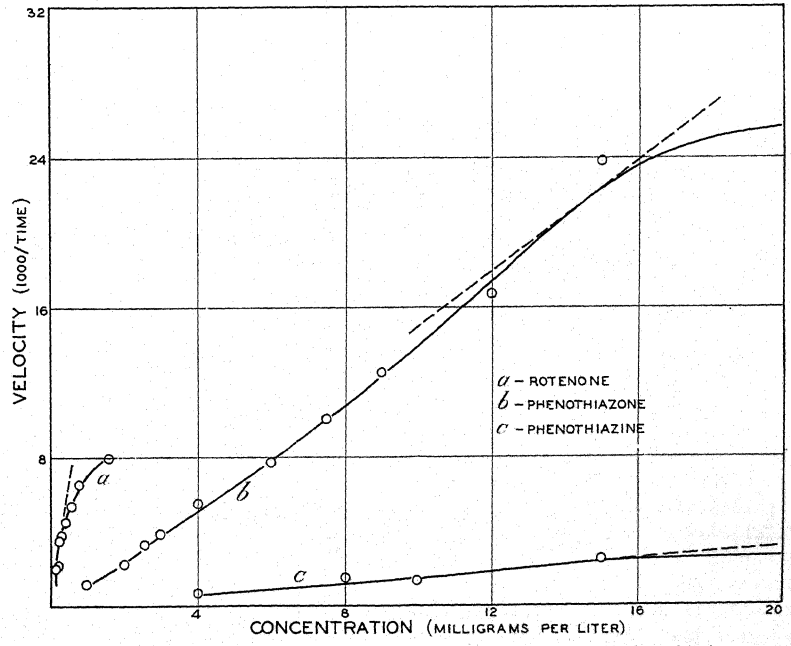


FIGURE 2.—Velocity-of-fatality curves for phenothiazone, rotenone, and phenothiazine.

TABLE 1.—*Toxicity of phenothiazine and its oxidation derivatives and of rotenone to goldfish at 27.0° ±0.2° C.*

Compound and concentration [1] (milligrams per liter)	Fishes used	Mean length of fishes	Mean weight of fishes [2]	Mean survival time [3]		1,000÷geometric mean survival time (velocity of fatality)
				Arithmetic	Geometric	
Phenothiazine:	*Number*	*Millimeters*	*Grams*	*Minutes*	*Minutes*	
50.0	2	65	7.5	ca. 360	ca. 360	2.78
15.0	9	[4]	[4]	>450	>400	<2.50
10.0	10	61	6.6	860±110	730 × 1.18	1.37
8.0	8	57	5.5	730±100	660 ÷ 1.13	1.52
4.0	8	[4]	[4]	>1,500	>1,500	<.67
1.0	12	[4]	[4]	[5] (48 hours)		
Phenothiazine sulphoxide:						
50.0	3	[4]	[4]	(27 hours)		
10.0	3	[4]	[4]	(48 hours)		
Thionol:						
50.0	3	[4]	[4]	(23 hours)		
10.0	10	[4]	[4]	(30 hours)		
1.67	10	[4]	[4]	(30 hours)		
Phenothiazone:						
50.0	2	59	6.0	40±3	40 × 1.07	25.0
15.0	10	59	6.0	43±3	42 × 1.07	23.8
12.0	9	56	5.3	61±2	60 × 1.04	16.7
9.00	10	58	5.8	82±3	80 × 1.04	12.5
7.50	10	57	5.4	101±3	100 × 1.03	10.0
6.00	10	59	6.0	131±3	130 × 1.03	7.69
4.00	11	56	5.2	183±3	182 ÷ 1.02	5.49
3.00	10	58	5.7	259±7	257 × 1.03	3.89
2.57	9	60	6.3	307±17	299 × 1.05	3.34
2.00	12	59	6.0	480±38	446 × 1.09	2.24
1.00	12	58	5.7	940±77	874 × 1.08	1.14
Rotenone:						
1.600	13	62	6.8	130±6	127 × 1.04	7.87
0.800	19	57	5.5	157±5	155 × 1.03	6.45
0.600	25	58	5.8	202±10	187 × 1.06	5.35
0.400	17	58	5.8	239±15	223 × 1.07	4.48
0.300	34	58	5.6	283±12	266 ÷ 1.04	3.76
0.225	6	57	5.3	306±39	282 × 1.14	3.55
0.200	10	62	6.8	480±35	457 × 1.07	2.19
0.150	6	57	5.5	521±63	486 × 1.14	2.06
0.075	8	57	5.3	>700	>700	<1.43
0.050	12	[4]	[4]	(31 hours)		

[1] As the first three compounds are insoluble, the figures signify the quantity added to the water; the last two compounds form true solutions.
[2] Estimated from length, which measurement excludes the tail.
[3] The limits of error indicated are probable errors of the means.
[4] Fishes not measured or weighed but of same approximate size.
[5] Figures in parentheses indicate that the material was not lethal in the time indicated.

Since in this case the two types of curves have reciprocal relationship, interpolation was possible. Therefore, the velocity-of-fatality curve was drawn from selected points on the more readily drawn survival-time curve, especially in the neighborhood of a critical point to be discussed later.

The test suspension of phenothiazine of 50 mg per liter, after exposure to the air and light for several days, acquired a pinkish color similar to that of phenothiazone at a concentration of about 1 mg per liter. So slight an increase in a toxic effect already but poorly defined could not be demonstrated with the small number of fishes permitted.

The test suspension of phenothiazine sulphoxide of the same concentration acquired in a few days a deeper color similar to that of phenothiazone at a concentration of 4 to 5 mg per liter. In the same time appreciable toxicity had developed, two fishes being killed in 140 minutes. Just before this test the suspension was aerated for half an hour, to avoid any toxic effect due to depletion of oxygen. During the test there was no evidence of oxygen hunger on the part of the fishes.

It therefore appears that, whereas phenothiazine sulphoxide may change appreciably on exposure to air to a compound coloring solutions similarly to phenothiazone and about as toxic, such a change in phenothiazine is slight. Within an organism, however, conditions for oxidation may be better than in this simple test, the toxic effect being limited only, as in the case of the goldfish, by the "invadability" of the compound.

DISCUSSION OF RESULTS

It is apparent at once from the curves in figures 1 and 2 that phenothiazone is appreciably less toxic than rotenone. The quantitative ratio depends somewhat on the method used in making the comparison, as emphasized in previous papers (*4*, *6*). A method that compares toxicity in the range of most efficient action is the comparison of the minimum products of concentration and survival time (*4*). These values are calculated directly from the survival-time curves. They may, however, be determined geometrically from the velocity-of-fatality curves by drawing the maximum tangents to the curves from the origin, as indicated in figure 2. In the latter case the slope of these lines, expressed in the units of the two variables, will give the reciprocals of the minimum products. The data for comparison by this measure are given in table 2. The minimum product of concentration and survival time is designated by $c_m t_m$ and, since toxicity varies inversely with this value, its reciprocal is also given. Its approximate coordinates c_m and t_m are given so that the region of the curve fulfilling this condition may be located readily.

TABLE 2.—*Relative toxicity to goldfish at 27° C. of phenothiazine, phenothiazone, and rotenone*

Compound	c_m	t_m	$c_m t_m$	Toxicity, $\frac{1}{c_m t_m}$	Relative toxicity
	Milligrams per liter	*Minutes*	*Gram-minutes per liter*	*Liters per gram per minute*	
Rotenone	0.26	300	0.078	13	1.0
Phenothiazone	14	48	.67	1.5	.11
Phenothiazine	12	500	6.0	.17	.013

These results show that, when compared at the range of most efficient toxic action to goldfish of this size at 27° C., rotenone is nearly 10 times as toxic as phenothiazone, and the latter is approximately 10 times as toxic as phenothiazine. It must be stressed, however, that the phenothiazine is not wholly in solution, and because of the wide distribution of results the values give only an indication of the relative toxicity under these conditions of suspension. Likewise, the insolubility of phenothiazine sulphoxide and thionol may preclude any toxic action under these conditions.

The fact is significant that, on standing, the test suspensions of phenothiazine and phenothiazine sulphoxide acquired a pinkish color similar to that of phenothiazone solutions, accompanied by an increase in toxicity. This change was much the greater in the case of the sulphoxide. In addition such suspensions, as well as the filtered colored solutions, showed a delayed toxic action, as indicated by the fact that fishes taken from incomplete tests in an active upright condition

and placed in fresh water later died. It therefore appears likely that these two compounds may oxidize to a more toxic compound, which may be phenothiazone.

SUMMARY

A study was made of the toxicity with respect to concentration and survival time at 27° C. of phenothiazine, phenothiazine sulphoxide, phenothiazone, and thionol, and the results were compared with those for rotenone. Goldfishes of the same lot weighing approximately from 5 to 7 g were used as the test animals.

Phenothiazone dissolved completely, the other sulphur compounds but slightly, in water at the concentrations required.

The most toxic of the sulphur compounds under the conditions of the comparison was phenothiazone. According to the minimum product of concentration and survival time, which measures toxicity at its range of most powerful action with respect to these two variables, phenothiazone was one-tenth as toxic as rotenone, but was itself 10 times as toxic as phenothiazine.

Phenothiazine sulphoxide and thionol were not appreciably toxic to goldfish of this size.

Test suspensions of phenothiazine sulphoxide acquired appreciable toxicity when exposed to the air for several days and took on a reddish color similar to that of solutions of phenothiazone. Test suspensions of phenothiazine went through a similar change but to a lesser degree.

It appears probable, therefore, that the toxicity of phenothiazine to goldfish may be due to oxidation, after absorption through the gills, to a more toxic compound, which may be phenothiazone. In that case the toxic effect is small because of the insolubility of phenothiazine.

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A PORTABLE INSTRUMENT FOR THE ANALYSIS OF HYDROCYANIC ACID GAS-AIR MIXTURES[1]

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INTRODUCTION

From the inception of fumigation research in Florida, it has been felt that an apparatus giving instantaneous concentration readings of various fumigants during the fumigation process would be a boon to the worker in this field. Fumigation experiments have revealed that most of the harmful agricultural insect pests succumb to very low concentrations of hydrocyanic acid gas in air. The analyzer for use in studying the effects of HCN as an insecticide must, therefore, measure low concentrations of the gas (less than 1 percent) to within a few hundredths of a percent. A search of the literature failed to locate an instrument of this sort until mention of a simple device for measuring concentrations of HCN in air was found in a paper by Peters.[3] Details of this instrument were not given in the paper and subsequent correspondence brought word from the author that the apparatus was not practical. In the face of this, however, work was started on the apparatus to be described.

The instrument depends for its operation upon the presence of a combustible gas mixed with air, passing through a "cell" containing an electrically heated platinum wire. If the current in the wire and the flow of the gas mixture through the cell are kept constant, any combustion of the gaseous mixture taking place at the surface of the platinum wire will cause a definite increase in the resistance of the wire which can be detected by means of a Wheatstone bridge.

THE CELL

It was found by experiment, using a potentiometer, that No. 36 platinum wire was the smallest gage filament that could be used and maintain consistent results, and that a heating current of approximately 1.4 amperes was required to heat the wire to the combustion point of the gas mixture. At this temperature the wire glows a dull red. Accordingly the cell was designed to carry 7 cm of No. 36 platinum wire giving approximately 2.5 volts drop when carrying 1.4 amperes of current.

Several shapes and designs of cells were tried before the one shown in figure 1 and described below was adopted. In this cell the heated filament is placed directly in the flow of gas; this is made necessary by the low concentrations of HCN used, and requires that the flow of gases be kept sensibly constant. The arrangement has the advan-

[1] Received for publication August 10, 1937; issued April, 1938.

[2] The writers acknowledge with gratitude the valuable assistance in the design of the electrical circuit rendered by L. W. Gaddum, biochemist, Florida Agricultural Experiment Station, and R. C. Williamson, professor of physics, University of Florida.

[3] PETERS, GERHARD. A SHORT GUIDE TO TREE FUMIGATION. [Transl. by M. Herbert.] Ed. 2, 191 pp., illus. Frankfort o. M. 1934.

tage, however, that no bulky cooling baths for the cell are required; the passage of the gas mixture through the cell carries away excess heat. To facilitate this cooling action, several turns of the copper tubing leading the gas mixture to the cell are wound around the cell and the unit is wrapped in asbestos paper to eliminate the effects of air currents.

The filament is zigzagged across the cell and welded to two lead-in wires of nickel-chromium alloy which are, in turn, soldered into two brass bolts passing into the cell through airtight bakelite sleeves. This end of the cell is threaded into the other half so that the filament is easily exposed if repairs are necessary.

The above-described cell departs somewhat from the type used in the Mine Safety Appliances Co.'s combustible gas indicator, and that of the Leeds & Northrup CO_2 recorder.[4]

GAS CIRCULATION

As mentioned above, the flow of gas past the filament must be maintained at a constant value, and in fumigation it is also necessary to draw the gas from some distance because it is unsafe for the operator to enter the space being fumigated. A flow of 1 liter per minute was found to give satisfactory sensitivity and steadiness of operation. To supply this circulation a positive rotary pump was constructed. Driven by a small 6-volt motor, the pump is capable of drawing a sample from a distance of over 100 feet through a ¼-inch copper tube at the rate of 1 liter per minute. The motor under normal load uses approximately 1½ amperes of current.

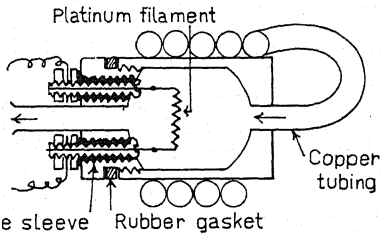


FIGURE 1.—Cell with heated platinum filament through which the gas flows.

It can be seen that the use of this instrument in connection with experimental work with fumigatoria is limited to those of the larger size because of the fact that it is necessary for the sampling flow to be continuous. At least, it should be to give satisfactory results. In a 100-cubic foot fumigatorium, however, the concentration would only be reduced six points in the third decimal place with a theoretical concentration of 0.3 percent HCN by volume. The instrument has been used in connection with a 20-cubic-foot box [5] with a flow of one-half liter per minute and it was calibrated accordingly. In this case there was no leakage and the volume decrease could be compensated for.

The sampling tube is connected to the instrument by means of a brass copper-tubing connection fastened into the instrument panel. This connector is drilled and tapped out to hold a small brass tube, one end of which is covered with fine copper screen to act as a flash-back arrestor. From this connector a copper tube carries the sample

[4] ROSECRANS, CRANDALL Z. AUTOMATIC GAS ANALYSIS RECORDER FOR THE RANGE OF 0 TO 3.5 PERCENT OF CO_2 IN AIR. Jour. Optical Soc. Amer. and Rev. Sci. Instruments 14: 479–490, illus. 1927.

[5] CAMP, A. F., and WILMOT, R. J. FUMIGATION RESEARCH IN FLORIDA. Fla. State Plant Bd. Monthly Bull. (1930–31) 15 (5–8): 1–35, illus. 1931.

to the flowmeter. This flowmeter is an adaptation of the usual type changed in form so that it can be read on a horizontal plane. It is blown from 1 mm capillary pyrex tubing, and in order to have the liquid come to the center or indicator mark on the arm which shows through the bakelite panel, it is necessary to adjust the size of the orifice and the volume of the liquid by trial and error until the correct combination is found (fig. 2). In order to make the orifice adjustable, a short length of copper tubing was flanged on both ends and a disk of platinum foil soldered into one end. The foil is pierced with a hole approximately 0.02 inch in diameter. Platinum was used to obviate corrosion and subsequent change in calibration of the flowmeter. The length of copper tubing is inserted into the flowmeter and is held in place by means of short lengths of rubber tubing, as shown in the diagram. The liquid used is kerosene, which has been treated with sulphuric acid to remove the unsaturated hydrocarbons and then colored with a little gasoline dye. Next to the flowmeter is the needle valve which is used to control the flow. It is a ¼-inch valve with copper tubing connections and a knurled knob replacing the usual T-type handle. From the valve the sample passes through copper and rubber tubing to the cell, then through a HCN gas-mask canister, and from it through a 1-pint expansion chamber to the pump. A small hole is drilled in the last length of tubing to let in extra air to compensate for the excess suction of the pump.

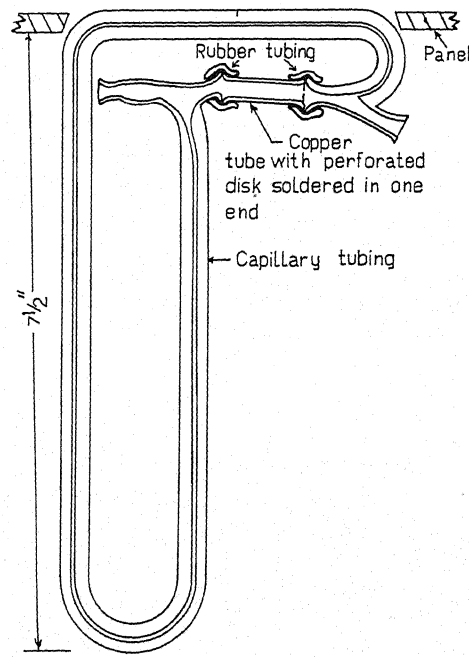


FIGURE 2.—The flowmeter.

The pump (fig. 3) is constructed of brass and steel with a brass rotor and a steel housing which is bolted directly to the motor housing. The rotor is approximately five-eighths of an inch thick and 1¼ inches in diameter, with six vanes set into it. These vanes are of composition similar to Bakelite one-sixteenth of an inch thick and three-eighths of an inch long and are set into the rotor at an angle of 30° to the radius; if rotation is clockwise they should point counterclockwise outward. The housing is bored out one-eighth of an inch off-center so as to be eccentric to the rotor. The inlet and outlet are spaced equally on each side of the point where the rotor and housing touch. A second hole is placed next to the inlet for an oil cup. The oil inlet must be very small, preferably adjustable so the amount of oil entering can be kept to a minimum, and the oil used should be of the lightest grade obtainable.

ELECTRICAL CIRCUIT

In the interest of portability, automatic current compensation and attendant bulky cooling baths have not been used. A 6-volt storage battery is necessary because of the heavy currents used by the bridge and air pump but, in the present-day use of automobiles, one is usually available nearby.

It was found impractical to give each of the bridge arms the resistance values called for by the resistance of the filament. Had this been done heavy current and correspondingly heavy resistance wire would have been necessary in both branches. The final compromise leaves the bridge symmetrical about an axis at right angles to the current and as nearly symmetrical about the other axis as is necessary to give proper sensitivity.

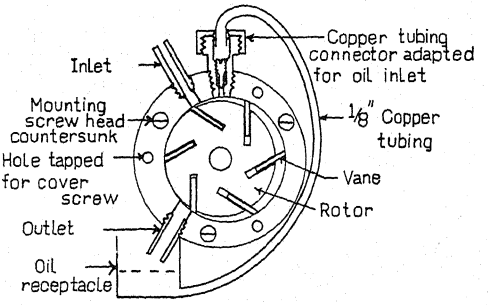


FIGURE 3.—The pump.

The current rheostats *j* (fig. 4) are a 1Ω and a 3Ω in parallel, the 3Ω being used as a fine adjustment on the 1Ω. These rheostats should be of a heavy-duty type capable of dissipating 250 watts under continuous load but should have sufficient windings to give smooth operation. Resistance *xc* is approximately 8 feet of No. 18 resistance wire rated at 0.18Ω per foot with a temperature coefficient of 1.002 for a change of 73° C. This is space-wound on a porcelain tube 1 inch in diameter, 5½ inches long, and is enclosed in an asbestos box. The slide wire *p* (position *dc*, fig. 4) is a 12-inch length of similar resistance wire mounted around the edge of a 4-inch bakelite disk, on the upper side of which is placed the calibrated card seen through a window in the bakelite panel. A shunt, in this case an equal length of the same kind of resistance wire, permits regulation of the effective resistance of the slide wire which, in the present design, is 0.115Ω. Flexible leads connect it to resistance *xc* and the filament *ef*. The brush on the slide wire is of the brass plunger type with a spring to maintain the tension against the wire. A piece of platinum foil is soldered to the end of the plunger to give a good contact surface. The lead from the brush is connected to one corner of a two-pole, double-throw switch (fig. 5, *A*, 6). The adjacent corner of the switch is connected between the 500Ω resistances *l* and *m* (fig. 4). These resistances are wire-wound and will not change with ordinary changes in temperature. The center poles of the switch are connected to a center-zero type galvanometer with a tapping key in the circuit. The galvanometer has a

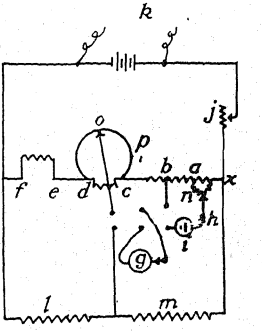


FIGURE 4.—Circuit diagram: Total amperage, 3.0a; filament amperage, 1.4a; resistance *xa*, 0.058Ω; resistance *xb*, 0.936Ω; resistance *xc*, 1.464Ω; resistance *cd*, 0.115Ω, filament *ef*, 7 cm of No. 36 platinum wire; *g*, galvanometer; resistance *h*, 50Ω; *i*, two 1½-volt batteries; resistance *j*, 1Ω and 3Ω rheostats in parallel; *k*, blower switch; resistance *l-m*, 500Ω each; resistance *n*, 200Ω; *o*, slide wire brush; *p*, slide wire.

sensitivity of approximately 2 microamperes per division and a resistance of approximately 125Ω.

The current checking circuit is standard. The span xa in $x+c$ (fig. 4) can be quite small, as its resistance need only be enough to allow com-

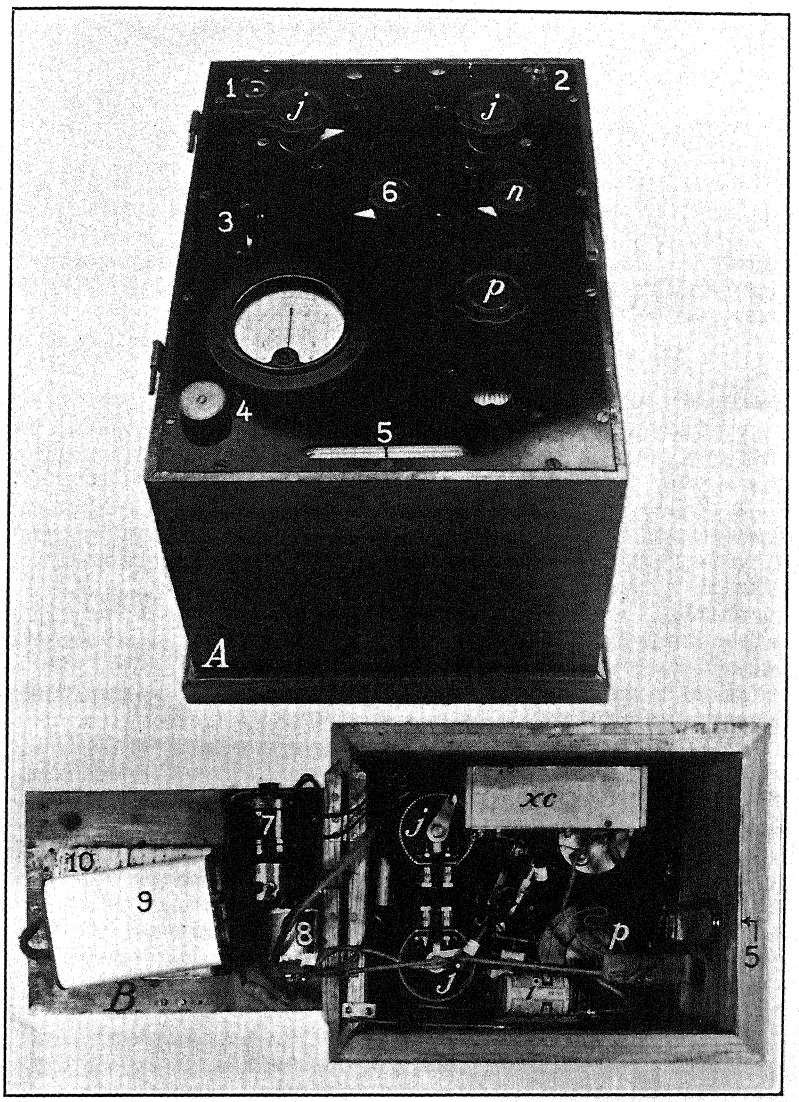


FIGURE 5.—*A*, Exterior of the analyzer: 1, Blower switch; 2, sampling-tube connection; 3, key in galvanometer circuit; 4, needle-valve knob; 5, flowmeter; 6, double-throw double-pole switch. *B*, Interior of the analyzer: 5, Flowmeter; 7, motor and pump; 8, cell; 9, expansion chamber; 10, gas-mask canister. Letters refer to letters on circuit diagram in fig. 4.

pensation for small variations in the zero balance of the circuit which will occur from time to time due to changes in temperature and relative humidity between experiments. The voltage divider shunt should have a resistance large enough to prevent an appreciable

current from flowing through it, and in this case is a 200Ω potentiometer rheostat (fig. 5, *A*, *n*, "CHECK"). It has a power rating of 5 watts. The brush on *p* is connected to the wire-wound resistance *h* (fig. 4), which is a 5-watt 50Ω resistance. The resistance of *h* determines the sensitivity of the check circuit; *h*, in turn, is connected to the positive pole of two 1½-volt flashlight cells (*i*) in parallel. A standard cell could be used at this point for greater accuracy, with corresponding changes in *xa* and *xb* and *h*, but it was thought that the cost was prohibitive. The negative pole of the battery (*i*) is connected to one of the remaining corners of the double-throw switch and the last corner of the switch is connected to the point *b* on resistance *xc*. The value of *xb* is approximately 0.936Ω. It was found that, in determining the proper position of connection *b*, if the battery connections were properly made, that is, with the positive pole of the storage battery to *j*, the positive pole of *i* to *xa*, and the + terminal of the galvanometer to *i*, when the galvanometer needle throws to the left, *b* should be moved toward *c*, and if to the right, toward *x*.

OPERATION OF THE INSTRUMENT

Before the pump is started the bridge is allowed to "warm up." This warming up begins as soon as the battery connections are made and is necessary because of the relatively heavy currents employed. The sampling tube is connected to the instrument and placed in the area to be sampled before the gas is released. After 15 or 20 minutes, when a temperature equilibrium has been reached, the pump may be switched on, and the flow adjusted to 1 liter per minute by opening the needle valve until the liquid in the flowmeter is brought to the indicator mark. With the dial of the slide wire *p* (fig. 5, *A*) set at zero, and the double-throw switch in the "Read" position, the galvanometer is brought to zero by means of the current rheostats *j*. When there is no further change the switch is thrown to the "Check" position and the galvanometer brought to zero by means of the potentiometer rheostat *n* ("CHECK"). After this setting is once made it is not changed during the course of the run, and the current rheostats are used to bring the current to the value determined by the initial "Read" setting with dial at zero.

The HCN is then released and readings are taken at any desired interval directly off the slide-wire dial, first making sure the flow is correct, by throwing the switch to "Check" and bringing the galvanometer to zero by means of the current rheostats, and then throwing the switch back to "Read" and bringing the galvanometer to zero by means of the slide-wire *p*. The reading is then taken off the slide-wire dial. This whole operation can be done in less than a minute. If the concentration is changing rapidly it is best to use the average of two readings. The arrangement of the controls is shown in figure 5.

The sampling tube has to stay in its initial position because the initial settings made were for the particular conditions under which the fumigation was being made and they compensate for the changes that different combinations of temperature and relative humidity might have on the readings. This was thought to be simpler than to calibrate the instrument under standard conditions and then work out correction tables for various combinations. If a number of points in a fumigatorium are to be sampled, there is no reason why the instrument cannot be connected to a manifold and gas be selected from any of

several points by a system of valves. Time would have to be allowed for the tube just coming into use to be thoroughly flushed out with the gas before readings were taken, but with a flow of a liter per minute this would only be momentary.

The instrument cannot be set to zero outside of the space being fumigated unless the operator is sure that the conditions are the same as they are inside. When this is the case, there are no objections to doing so. It is possible that, in orchard fumigation, for instance, the instrument could be moved from tent to tent because the conditions would not change materially from one tent to another.

When the instrument is used in a glass system such as Allison's,[6] having means for measuring and controlling atmospheric pressure, temperature, and relative humidity, the flow is started through the system and the instrument brought to a balance following the usual procedure. The system must be flushed out for some time after use so that there will not be any residual HCN to cause an error in the setting. The flowmeter in such a system can be used to calibrate the flowmeter in the instrument. The flowmeter, pump, gas-mask canister, etc., in the instrument are of course unnecessary when such a system is used because the flow can be maintained by the other means.

CALIBRATION

Calibration readings were taken in two ways: One while the analyzer was connected into a glass system such as is mentioned above, having means for measuring and controlling temperature, relative humidity, and atmospheric pressure, and another in which the sample was drawn from a fumigatorium while it was in operation under actual field conditions. In each case chemical analysis, by a variation of the Liebig method,[5] provided the concentration data used in calibrating the slide-wire settings of the analyzer. It was found that, by having the check circuit adjustable by means of the potentiometer n, a calibration would hold for a temperature range of 10° C. (from 20° to 30°) and a range of relative humidity from 20 percent to 90 percent. The concentration readings obtained with the instrument are accurate to about ± 0.02 percent HCN by volume under field conditions. The calibration was made in percentage by volume since the method of chemical sampling employed volume ratios. Figure 6 is a sample calibration curve in which the chemical analyses have been plotted against readings from an arbitrary linear scale on the slide-wire dial. Using the calibration curve, a concentration scale was marked on a circular card and attached to the slide wire p.

APPLICATION

The instrument described above will analyze hydrocyanic acid gas-air mixtures accurately under most conditions under which fumigation is carried on. It can, therefore, be used to determine whether or not buildings, holds of ships, etc., can be safely entered. It can also be used to determine whether or not the fumigation is being carried on efficiently, because, after all, it is the concentration of the gas reaching the insect that effects the kill.

It is believed that the instrument may be modified for use with other fumigation gases such as carbon bisulphide, since preliminary experi-

[6] ALLISON, JAMES B. STUDIES ON THE TOXICITY OF HYDROCYANIC ACID. Iowa State Coll. Jour. Sci. 2: 243–252, illus. 1928.

ments show a definite response of the instrument to low concentrations of CS_2.

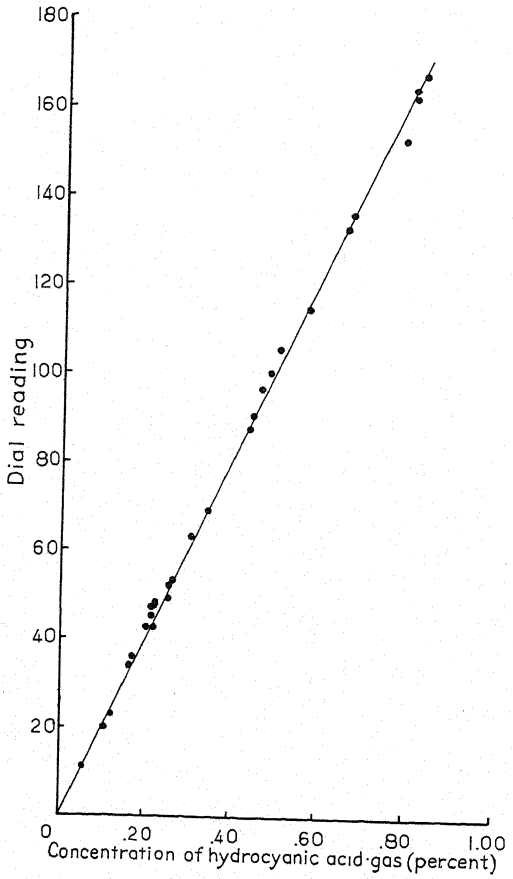


FIGURE 6.—Sample calibration curve in which chemical analyses for HCN were plotted against readings from an arbitrary linear scale on the slide-wire dial.

SUMMARY

An instrument which analyzes hydrocyanic acid gas-air mixtures by physical apparatus is described. The sample is pumped from the space containing the gas by means of a small motor-driven pump contained in the instrument, and passed over a hot platinum filament; the change in resistance of the filament is then measured by means of a bridge, and translated into percentage of HCN by volume. Its accuracy is ± 0.02 percent HCN by volume.

FUNGUS GROWTH IN SHELLED CORN AS AFFECTED BY MOISTURE[1]

By BENJAMIN KOEHLER

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INTRODUCTION

Infection of corn caused by ear-rot fungi begins soon after pollination and may continue until stopped either by lack of moisture in the grain or by low temperature. Sometimes these ear-rot fungi cause considerable damage after the corn is cribbed or shelled and placed in storage. The study here reported was made to determine at what moisture limits growth of ear-rot fungi may take place at a temperature suitable for their development. To facilitate study, all the grain used in the experiment was first shelled from the cobs. A number of aspergilli and penicillia became conspicuous in the corn stored under controlled conditions and these were included in the study. These forms have often been recognized as important storage-rot fungi by other workers. They have not been observed by the writer to cause significant losses to corn ears prior to harvest except that they often produce moldy areas where the ears have been injured by birds or insects. Short progress reports of this work have been published (*9*, *12*).[2]

METHODS

STORAGE CHAMBERS

Two styles of storage chambers were used. When no disinfectant was applied because pure culture work was not desired, the shelled corn (*zea mays* L.) was placed in cylindrical wire baskets holding 100 g and hung over salt solutions in wide-mouthed half-gallon glass bottles. In most of the experiments the bottles were supplied with glass tubes 15 cm long with a 3-mm bore which were inserted through the rubber stoppers that closed the bottles. This supplied aeration sufficient to prevent the sour alcoholic odor which developed on corn above a certain moisture content in chambers entirely sealed, and at the same time checked appreciable evaporation. In fact, under sufficiently limited aeration the production of metabolic water by respiration seems to compensate fully for that lost by diffusion.

For pure culture work with a single fungus the apparatus shown in figure 1 was used. This method, adapted from Shippy (*15*, *pp. 370–372*) and Hatfield (*7*), provided a constant humidity and fresh sterile air. Before the surface-sterilized shelled corn, was introduced the entire apparatus was sterilized under steam pressure. Some of the rubber tubes were disconnected during the autoclaving process to prevent the solution from being forced from one flask into another. An additional flask of salt solution at first used in the chain was found

[1] Received for publication July 19, 1937; issued April 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 306.

unnecessary. A battery of 12 of these sets was operated at one time in a constant-temperature chamber. After the first few days of more thorough aeration, the air flow was adjusted to one bubble in 2 or 3 seconds.

HUMIDITY CONTROL

While partial saturations of sulphuric acid are commonly used for humidity control where the relative humidity must be known (*17*, *22*), in this case results were recorded in terms of moisture content of corn, and therefore a series of partial concentrations of any one of a number of salts would answer, and calcium chloride was chosen for use in the half-gallon bottles. A series of concentrations from 24 to 0 percent produced atmospheric humidities which caused the corn moistures to range from 14 to 29 percent.

In the sets where air was bubbled through the solutions, saturated solutions of a variety of chemical compounds were used. The chemicals used are given in table 1. They are arranged in ascending order

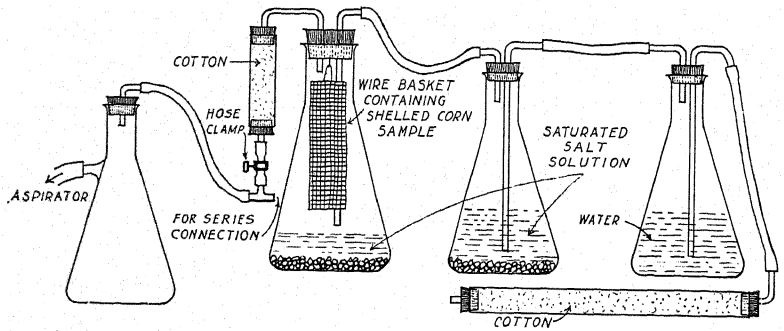


FIGURE 1.—Apparatus used for storing corn under constant-humidity conditions with a slow uniform change of air, and for protecting it from contamination with micro-organisms.

with respect to corn moisture, and therefore should also be in ascending order with respect to relative humidity. The values for relative humidity as compiled by Spencer (*16*) (table 1) are not in full agreement with this. Loss of water from a saturated solution does not affect the relative humidity over the solution. In actual use it was found that after a 3-month run, only a little water need be replenished in the end flask and there was no appreciable change in volume in the flasks containing chemical solutions.

Over some salt solutions, within certain limits, relative humidity is affected very little by temperature, while over others it is affected considerably. Furthermore, in either case a sudden change in temperature would temporarily throw the humidity out of balance, and at high humidities the dew point is easily reached, not only on the inner surface of the flask but also within the corn sample. Therefore, storage was carried out under constant temperature arbitrarily chosen at 70° F.

CORN USED

Well-developed, mature Illinois station Reid Yellow Dent ears free from blemishes were selected and 10 kernels from each were tested on a tray type germinator. This gave an approximation of the kind of internal infection, if any, the ears carried. Disease-free ears and

ears having grain infected with only one fungus, *Diplodia zeae* (Schw.) Lév., *Fusarium moniliforme* Sheldon, *Gibberella zeae* (Schw.) Petch, *Nigrospora sphaerica* (Sacc.) Mason (=*Basisporium gallarum* Moll.), or *Cephalosporium acremonium* Corda were selected. The ears were further selected after surface sterilizing 20 representative kernels from each ear and plating on potato-dextrose agar. Air-dry corn ranging from 10 to 13 percent in moisture and with the desired amount of water subsequently added was usually used, but some checks were made by selecting ears fresh from the field which contained a higher percentage of natural moisture. Whether or not the corn had previously been dried apparently made no difference in the results. This is in agreement with the finding of Swanson (*18*) in work with stored wheat.

TABLE 1.—*Moisture content of unsterilized shelled corn after storing over saturated solutions of different chemicals in closed containers* [1] *for 12 weeks at 70° F.*

Name of chemical	Relative humidity, at 20° C.[2]	Tests made	Moisture content of corn	
			Range	Mean
	Percent	*Number*	*Percent*	*Percent*
Ammonium sulphate	81	4	15.2–16.2	15.7
Potassium bromide	84	6	15.3–17.6	16.5
Potassium hydrogen sulphate	86	13	17.1–19.7	18.2
Zinc sulphate	90	12	17.0–20.7	18.6
Sodium sulphate	95	11	17.9–21.3	18.8
Sodium sulphite	93	15	18.5–22.4	19.9
Barium chloride	88	13	19.0–21.8	20.2
Potassium nitrate	93	12	20.7–22.6	21.6
Sodium tartrate	91	4	21.0–22.2	21.7
Ammonium dihydrogen phosphate	93	9	21.0–23.4	22.2
Sodium bromate	92	16	21.5–23.5	22.3
Dibasic sodium phosphate	95	14	23.2–25.7	24.6
Oxalic acid	96	4	25.1–27.5	26.0
Potassium sulphate	97	7	25.3–27.2	26.2
Gypsum	98	5	27.2–30.1	28.7

[1] As shown in fig. 1.

[2] As reported by Spencer (*16*).

The corn moistures were determined by placing about 50 g of the grain in an open vessel 65 mm in diameter and drying at 100° C. for 4 days in an electric oven without vacuum. Percentages are based on weight of corn before drying.

SURFACE STERILIZATION

Where pure culture work was desired, the grain was sterilized on the surface with the filtrate from a fresh chlorinated lime solution. The time of soaking ranged from 15 minutes to 1 hour, depending on how much water it was desired to take up. A solution of 80 g of chlorinated lime per liter of water was used for the short-time soaks, with decreasing strengths for the longer soaks. The grain was treated in an open beaker with frequent stirring and then was transferred to a sterile wire basket and placed in the sterilized storage apparatus. Thorough aeration with sterile air to remove chlorine fumes and thus allow the fungi to develop was provided by the apparatus shown in figure 1.

SOME FACTORS AFFECTING THE AMOUNT OF HYGROSCOPIC MOISTURE TAKEN UP BY GRAIN

VARIABILITY IN CORN STRAINS

Corn from different ears placed over the same solutions did not always come to equilibrium at the same grain moisture content (table 1). An experiment conducted with several distinctly different types of dent corn revealed significant differences in the extent to which water was taken up by the different types from two different atmospheres (table 2). Two cubic centimeters of formalin was added to 198 cc of water in the bottom of the jars to prevent mold growth on the kernels, and the corn used was selected for freedom from internal infection. The formalin solution proved unstable; it gradually lost its disinfecting power and, therefore, was replaced once a month. The largest quantity of moisture was taken up by the old type Reid Yellow Dent corn, which is a moderately rough "starchy" type. The Illinois high- and low-oil and high- and low-protein strains (*10*) differ markedly with respect to oil and protein content. All the hybrids used were of a distinctly horny dent type and the moistures at the close of the test were definitely lower than in either one of the two kinds of Reid Yellow Dent. It was shown by Alberts (*1*) that starchy corn takes up hygroscopic water more rapidly than horny corn. Bailey (*3*) found differences in three varieties of corn with respect to their capacity for taking up hygroscopic water.

TABLE 2.—*Moisture in 10 strains of dent corn when the shelled grain was stored free from mold in two constant atmospheric humidities in sealed half-gallon bottles for 105 days at 70° F.*

Kind of corn	Over 10-percent $CaCl_2$ solution containing 1 percent formalin					Over pure water containing 1 percent formalin				
	Moisture of grain in each of 4 replicates				Average moisture	Moisture of grain in each of 4 replicates				Average moisture
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Station strain Reid Yellow Dent	20.5	20.8	20.0	20.3	20.4	29.0	29.1	28.9	29.1	29.0
Old type Reid Yellow Dent	21.5	21.5	21.0	21.4	21.4	29.9	30.2	30.1	29.7	30.0
Illinois high protein	19.3	19.2	19.3	19.7	19.4	27.8	28.0	27.9	27.7	27.9
Illinois low protein	20.7	20.7	19.7	20.1	20.3	28.9	29.6	27.8	28.5	28.7
Illinois high oil	19.3	19.2	19.0	19.4	19.2	26.8	27.4	27.0	27.3	27.1
Illinois low oil	19.8	20.2	19.6	20.0	19.9	29.4	29.3	29.2	29.5	29.4
Hybrid A5 × Hy [1]	19.9	20.0	19.2	19.4	19.6	27.5	28.0	27.0	27.2	27.4
Hybrid A × K [1]	19.3	19.3	19.3	19.1	19.3	26.5	26.9	26.4	26.4	26.6
Hybrid R126 × JL [1]	19.9	19.9	19.4	19.8	19.8	27.0	27.3	26.5	26.6	26.9
Hybrid (A×L) (Hy×R4) [1]	19.2	19.0	19.7	19.4	19.3	26.7	26.9	26.1	26.8	26.6

[1] Supplied by J. R. Holbert, formerly with U. S. Department of Agriculture.

CONDITION OF GRAIN

The amount of moisture taken up also is influenced considerably by whether the grain is moldy or free from mold (fig. 2). The range or variability in corn moisture over each of the solutions given in table 1 is a combined effect of differences in corn used and differences in moldiness and kind of mold used in the different tests. Alberts (*2*) found that removal of the seed coat from the crown of corn kernels affected the speed with which hygroscopic water was taken up

or lost, but experiments by the writer indicated that this had no effect on the ultimate moisture content of the grain after equilibrium with the surrounding atmosphere was reached, nor did previous killing of the grain by low temperature affect this relationship.

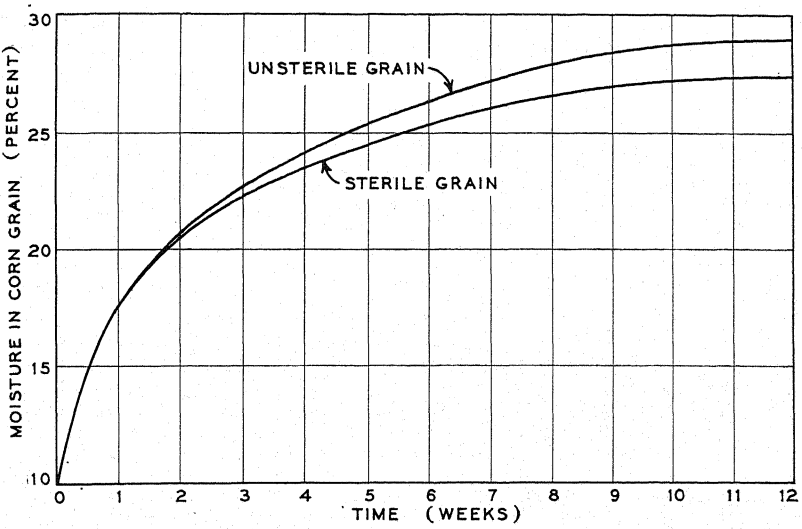


FIGURE 2.—Rise of hygroscopic moisture in shelled corn stored in a saturated atmosphere in closed containers for 12 weeks at 70° F. Grain from the same ears was divided into two lots; one was placed over plain water and molds developed; the other was placed over water containing 1 percent of formalin and remained sterile.

TIME AND TEMPERATURE OF STORAGE

At constant humidity and temperature, corn grain comes to equilibrium with the surrounding atmosphere very slowly, coming close to it in 12 weeks' time at 70° F., as shown in figure 2. To shorten this lag, sufficient water was added to the corn to bring it close to the desired moisture before placing it in the constant-humidity chambers, and a storage period of 12 weeks was chosen. Further fungus growth at low moistures might have been found if the storage period had been longer, for fungus growth is exceedingly slow when near the critical moisture (*18*, *23*). From a practical standpoint, on the other hand, it must be considered that the moisture of corn in storage is seldom static, and when it is above the critical point for mold growth there usually is a tendency for the moisture to decrease slowly.

The temperature chosen, 70° F., also has an important bearing on the results obtained. Swanson (*18*), for instance, found that wheat stored for 13 weeks at 95° F. was safe from mold growth only below 14 percent moisture content, but at 60° it was safe for the same length of time at 17.4 percent.

FUNGUS GROWTH IN RELATION TO MOISTURE OF GRAIN

ASPERGILLUS SPECIES

Members of the *Aspergillus glaucus* group [3] grow at a lower moisture content of grain than any other kind of fungi observed in these

[3] Some isolations of the *A. glaucus* group and *A. versicolor* were identified by Charles Thom, of the Bureau of Plant Industry, U. S. Department of Agriculture.

experiments. Some members of the group produced primarily conidia on the grain as well as on culture media while others produced primarily perithecia, as shown in figure 3, *A* and *B*. The green conidial heads were observed with the naked eye at moistures as low as 14.3

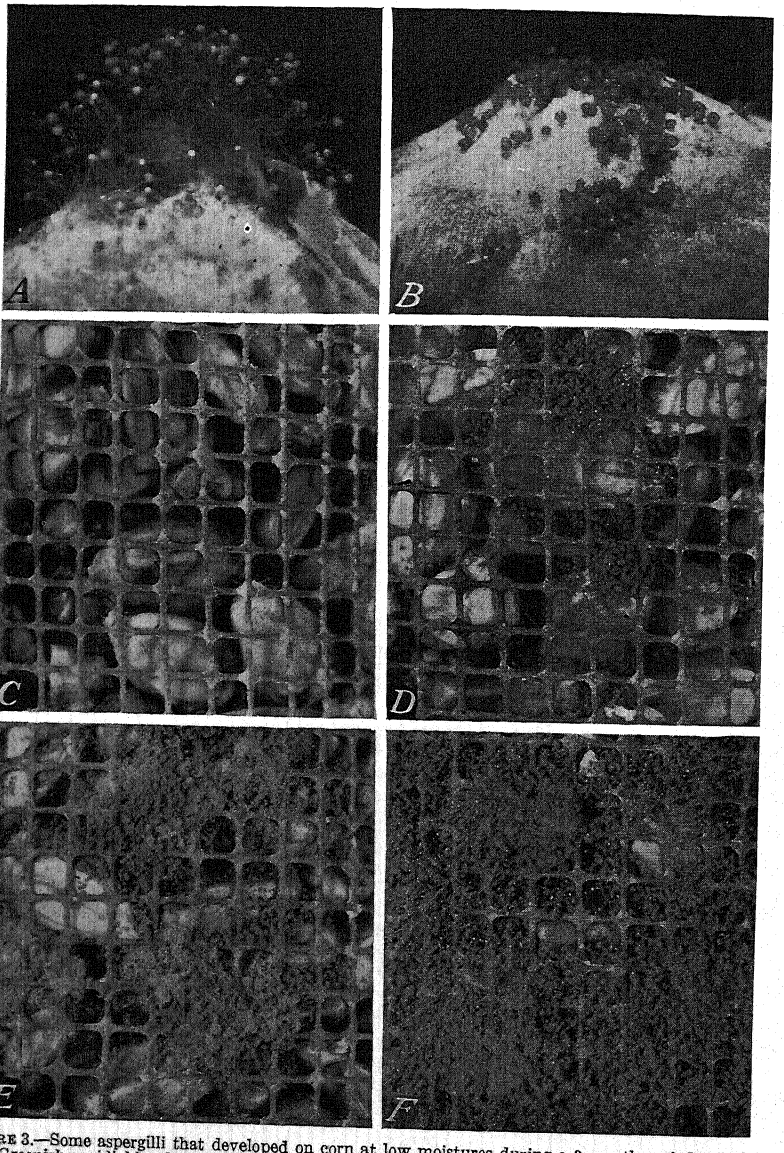


FIGURE 3.—Some aspergilli that developed on corn at low moistures during a 3-month period at 70° F.: *A*, Greenish conidial heads of *Aspergillus glaucus* growing on the tip end of grain with 15.5 percent moisture. *B*, Another form of *A. glaucus* producing bright-yellow perithecia on grain at 16 percent moisture. Both × 15. *A. glaucus* occurred at moistures as low as 14.3 percent. *C*, *D*, *E*, and *F*, *A. wentii* growing at moistures of 15.9, 16.8, 18.0, and 19.2 percent, respectively. × 2.

percent after 12 weeks' storage, as shown in table 3, and bright-yellow perithecia appeared at a moisture percentage only slightly higher. Thom and Le Fevre (*20*) found members of the *A. glaucus* group to

be the first molds active in corn meal as the moisture rose above 12.5 or 13 percent. This was a somewhat lower moisture than that found in the present experiments with whole grain.

TABLE 3.—*Critical moisture content of shelled grain of Reid Yellow Dent corn at which certain fungi were able to grow*

Name of fungus	Source of fungus	Tests made	Highest moisture at which no fungus growth was observed	Lowest moisture at which some fungus growth was observed
		Number	*Percent*	*Percent*
GRAIN NOT SURFACE-STERILIZED, COMPETITION BY OTHER ORGANISMS				
Aspergillus glaucus group	Natural infection	12	14.8	14.4
A. wentii	do	2	15.2	15.1
Penicillium spp	do	9	17.3	16.3
A. flavus	do	3	18.5	18.3
A. niger	do	12	20.8	18.3
Fusarium moniliforme	do	14	21.6	18.8
Diplodia zeae	do	9	22.4	21.2
Gibberella zeae	do	3	22.9	22.2
Nigrospora sphaerica	do	7	24.8	23.6
GRAIN SURFACE-STERILIZED, PURE CULTURE OF FUNGUS PRESENT				
A. glaucus group	Inoculation	2	14.0	14.3
A. versicolor	do	2	14.2	15.0
A. wentii	do	1	14.5	15.4
A. ochraceus	do	2	14.5	15.6
P. notatum	do	3	15.0	15.6
P. viridicatum	do	2	16.8	17.6
P. palitans	do	3	17.6	18.0
A. flavus	do	2	18.0	18.3
F. moniliforme	Natural infection	8	20.7	18.4
A. tamarii	Inoculation	1	18.7	19.8
A. niger	do	2	19.1	20.1
P. oxalicum	do	2	19.0	20.8
P. expansum	do	1	20.1	20.8
D. zeae	do	2	21.0	21.9
G. zeae	do	3	21.2	22.3
N. sphaerica	do	3	21.1	22.5
Cephalosporium acremonium	Natural infection	1	22.1	23.4

Aspergillus versicolor, *A. wentii*, and *A. ochraceus* developed at moistures only slightly higher than *A. glaucus* (table 3). *A. flavus* was not observed until a moisture content of 18.3 was reached, although it has been reported (*20*) as occurring in corn meal at a moisture as low as 16 percent. *A. niger* was found on grain with moistures of 18.3 percent and upward, while *A. tamarii*, observed in one test only, occurred at 19.8 percent. All of these species were observed growing naturally on unsterilized corn used in the experiments, but the lower limit for growth could not always be determined in that way. Some of the fungi that were observed were, therefore, isolated in pure culture and inoculations were made on surface-sterilized corn. No effort was made to determine the number and identification of all the fungi occurring naturally at the different moistures. In small grains *A. niger*, *A. flavus*, and *A. fumigatus* have been reported growing at 18 percent moisture (*6*). In another report (*14*) *A. glaucus* and *A. albus* were mentioned, the latter developing somewhat later and apparently requiring more moisture than *A. glaucus*.

Fungus growth at the lowest moistures occurred at the tip ends of the kernels where they had been attached to the cobs and at places where the seed coat was broken. At slightly higher moistures by close inspection a sparse amount of mycelium could be seen trailing through the spaces between the kernels, as shown in figure 3, *C*, and the formation of conidial heads was not limited to the places just mentioned. Different species showed some differences in growth habits.

PENICILLIUM SPECIES

Penicillium was found growing on the tips of corn kernels at moistures as low as 16.3 percent. By inoculating with some known cultures, the arrangement of species with respect to moisture requirement in ascending order was *P. notatum*,[4] *P. viridicatum* (in one of its forms),[4] *P. palitans*,[4] *P. oxalicum*,[5] and *P. expansum*.[6] The first three named were isolated from "blue eye" corn about which more is given below. *P. oxalicum* occurs commonly on corn ears before harvest when kernels have been mechanically injured by corn earworms or birds.

Penicillium expansum was included because McHargue (*14*) reported it as making more prolific growth than any of the molds observed in his corn-storage experiments. However, the culture of *P. expansum* used by the writer made the least vigorous growth on corn of any of the penicillia used. This difference in results may very likely be explained by differences in physiologic behavior of different strains of *P. expansum*. There is the question also whether *P. expansum* is considered in the broad sense in which some forms may be difficult to distinguish from some members of the *P. viridicatum* series, or whether the species is limited to that organism which, in addition to answering the morphological description, causes rot of apple.

A special study was made of the condition known to the grain trade as "blue eye" (fig. 4). It is caused by the growth of blue penicillia between the germ and the seed coat. Through the courtesy of W. B. Combs and H. P. English, of the Bureau of Agricultural Economics United States Department of Agriculture, samples of blue eye were obtained from the markets at Chicago, Milwaukee, Minneapolis, Cedar Rapids, Peoria, Toledo, and Nashville. Representative kernels but with unbroken seed coats were selected from each lot. They were surface-sterilized with a chlorinated lime solution, and the seed coat covering the germ was then opened and a transfer of the fungus spores made to sterile water. This was followed by the pouring of agar dilution plates. Sometimes the colonies obtained in the plates from a transfer from a single kernel appeared to be all alike; sometimes two different kinds of colonies developed in abundance, and occasionally there were three different kinds. All the isolations were penicillia. To make more sure of their purity, a needle transfer was made from the one or from each of the several kinds of colonies isolated from each kernel and a second set of dilution plates was poured.

After spending a whole winter season isolating, purifying, culturing and classifying these penicillia, and studying the keys and descriptions (*19*), the writer felt that he could not definitely name any of them

[4] Identified by Thom.

[5] Received from Helen Johann, of the Bureau of Plant Industry, U. S. Department of Agriculture, who had it identified by Thom.

[6] Received from H. W. Anderson, University of Illinois, who isolated it from a rotting apple.

unless he were to make a long-time study of the whole *Penicillium* group and secure an abundance of authentic specimens. As this was not the main purpose of the investigation, such a study was not attempted. Many of the isolations could be classified into three large groups within each of which there was a great deal of similarity. A fourth group was composed of various types which did not classify into the first three. No further work was done with the last group. Representative samples of the first three groups sent to Dr. Charles Thom in the spring of 1935 and again in 1936 were identified, while others were said to be mixtures. From the results of these identifications it appeared that for the most part one group represented members of the *P. chrysogenum* series, one group was *P. palitans*, a

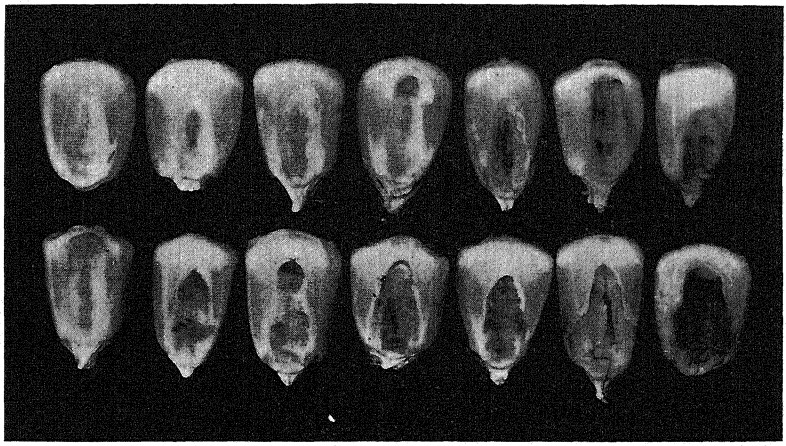


FIGURE 4.—Blue eye of corn caused by a growth of *Penicillium* over the germ and beneath the seed coat on grain with moistures of 18.5 to 24.0 percent. These specimens were received from Federal grain inspectors. Very little blue eye has been observed above the moistures given, for at higher moistures other molds usually predominated over the penicillia.

member of the *P. viridicatum* series, and the other group was composed of members of the "Fasciculata," ranging, according to Thom, "from *P. cyclopium* through the *viridicatum* series."

The lower moisture limits for the development of blue eye was determined by (1) observing its natural development from surface-borne spores, and (2) by inoculating surface-sterilized corn with *Penicillium notatum*, *P. palitans*, and with three different cultures belonging to the third group mentioned above. Corn not inoculated did not always develop blue eye even though moisture was adequate, but at times abundant blue eye developed. In the inoculated series *P. notatum* produced blue eye in 2 percent of the kernels at 16.7 percent moisture, in 10 percent of the kernels at 17.7 percent moisture, and in about 60 percent of the kernels at 19.0 percent moisture. *P. palitans* produced 10 percent of blue eye kernels at 19.5 percent moisture and about 50 percent at 21.2 percent moisture. The general appearance of the blue eye from the two inoculations was practically alike, being blue in color. The three unnamed species caused blue eye at about the same moistures as *P. palitans*, and one of them produced blue eye identical in appearance with it. Of the other two, one produced a grayish-blue color of the germ area, and the other produced a some-

what reddish or purple color. When kernels of the latter kind were placed in an alkaline solution the color became a bright blue. *P. oxalicum* and *P. expansum*, although growing and fruiting over the surfaces of the kernels, did not cause the typical blue eye condition.

At the lowest moistures, blue eye appeared mostly as a narrow stripe over the germ, as illustrated in figure 4, top row, second kernel from the left. This condition, called "hair line blue eye" by grain inspectors, in some forms is easily confused with the genetic condition known as purple plumule (*8*). When a kernel is cut crosswise through the plumule, however, the genetic purpling is seen to be in the tissue of the plumule, while the fruiting heads of penicillia causing the color of blue eye are located between the germ and the seed coat. With somewhat higher moistures blue eye becomes more general over the surface of the germ. At moistures above 23 to 26 percent, depending no doubt largely on what kind of fungi the grain is carrying, blue eye is likely to become obliterated by the growth of other organisms. Sometimes it is covered over by various aspergilli even at moistures below 23 percent. In natural, unsterilized stored shelled corn with moistures of 17 to 23 percent, sometimes the penicillia, sometimes the aspergilli, predominate.

FUSARIUM MONILIFORME

That there are wide differences in culture characters of *Fusarium moniliforme* is well known, and marked differences in pathogenicity to corn have been reported (*13, 21*). Differences were also found with respect to moisture requirements. Because of the variability in the results obtained, more tests were made with this fungus than with any other. Only naturally infected seed was used. Infection caused by *F. moniliforme* is the commonest of all internal seed infections in corn, and a great many kernels of each lot used in these particular tests carried this fungus. The term "pure culture" as applied to *F. moniliforme* in table 3 means only that no other kinds of fungi were present; most certainly there were a considerable number of strains of *F. moniliforme* in each lot of corn used and the growth at the lower moisture limit was determined by the particular strain that had the lowest moisture requirement. The 22 different experiments were conducted with 18 different lots of corn over a period of 8 years. Corn internally infected with other organisms of course was avoided in these tests.

In surface-sterilized grain growth of *Fusarium moniliforme* was seen at 18.4 percent moisture in one lot, while in another lot no growth occurred at 20.7 but growth did occur at 22.1 percent. In grain not surface-sterilized the end point for growth was more difficult to determine because some aspergilli and penicillia grow luxuriantly at moistures where growth of *F. moniliforme* is very feeble. However, by using a microscope and pouring dilution plates from needle-point transfers from suspected places, the presence of *F. moniliforme* could be definitely verified. In some of these tests the fungus was found to grow at moistures as low as 18.8 percent, while in others the grain was free from growth up to or above 21.6 percent. There seems to be no doubt that the variability of different strains of *F. moniliforme* was the principal cause of some of these differences. Furthermore, the strains used or observed probably do not represent the whole range,

for, as already pointed out, measurements were concerned entirely with those strains in each lot of corn that had the lowest moisture requirement.

Above 21 to 24 percent moisture *Fusarium moniliforme* grew vigorously, completely enveloping the grain with a powdery pink mass and with mycelium crisscrossing the spaces between the kernels. Frequently many of the germs became rotted and turned a deep reddish-purple color. In mixed cultures it sometimes was difficult to distinguish this discoloration from that produced by certain penicillia, although the usual type of blue eye produced by penicillia is decidedly bluish in color.

Commercial lots of corn examined over a period of years have always shown 10 percent or more infection from *Fusarium moniliforme*. Many infected kernels look normal and healthy. Two methods of testing have been used. (1) a tray germinator in which the corn is tested as received, and (2) plating the surface-sterilized grain on potato-dextrose agar. The percentage of infection has always been much higher by the first method than by the second, indicating that much of the infection was superficial enough to be killed by the disinfectant used. It is evident that most if not all lots of corn from the Corn Belt contain enough *F. moniliforme* infection to cause serious rot above 23 percent moisture if the temperature and oxygen supply are suitable. At this moisture *F. moniforme* will usually compete well and frequently will predominate over the aspergilli and penicillia, but if *Diplodia zeae* is present in sufficient quantity it, in turn, will predominate over *F. moniliforme*.

DIPLODIA ZEAE AND OTHER FUNGI

The moisture limits for growth of *Diplodia zeae*, *Gibberella zeae*, and *Nigrospora sphaerica* are very close together, as shown in table 3, and the general appearance of the three is very much the same (fig. 5). At 23.8 percent moisture *Diplodia* caused a dull dark discoloration of the germs and at slightly higher moistures all the kernels became tightly bound together by the white *Diplodia* mycelium. It grew aggressively and usually predominated over all other organisms.

Gibberella zeae had a pure white mycelium like *Diplodia* when grown at the lowest moisture limit, but at somewhat higher moistures the mycelium was pink in some areas and yellow and white in others, all colors occurring in the same basket of corn. At this moisture many of the kernels developed deep-red discolorations of the germs and other limited parts of the kernels. When the corn was not surface-sterilized, *G. zeae* did not usually predominate over aspergilli, penicillia, and *Fusarium moniliforme* until the moisture reached 26 percent and over. Above this moisture it became aggressive.

Nigrospora sphaerica grew as well as the two fungi just discussed when stored at corresponding moistures in pure culture (fig. 5). The mycelium was usually white but sometimes had a grayish appearance caused by the presence of spores. Some strains of *N. sphaerica* produced spores under the conditions of these experiments, while others produced none. The white parts of affected corn kernels turned a yellowish color at moistures allowing abundant growth of *Nigrospora*. When forced to compete with other fungi, this fungus grew poorly in stored corn containing less than 30 percent moisture. In three tests

with naturally infected grain, not surface-sterilized, and with moistures ranging up to 25 percent, *Nigrospora* could not be detected, whereas several other fungi were present in great abundance. In other similar tests some growth of *Nigrospora* could be detected by its characteristic spores at a moisture of 23.6 percent. The results obtained with *Nigrospora* infection in nonsterile seed are in agreement with some results reported by Durrell (4). Apparently this fungus is not ordinarily of importance as a cause of storage rot in corn even though it is present and there is sufficient moisture for its growth.

Cephalosporium acremonium infection in corn grain, while not ordinarily as frequent as that caused by *Fusarium moniliforme*, is never-

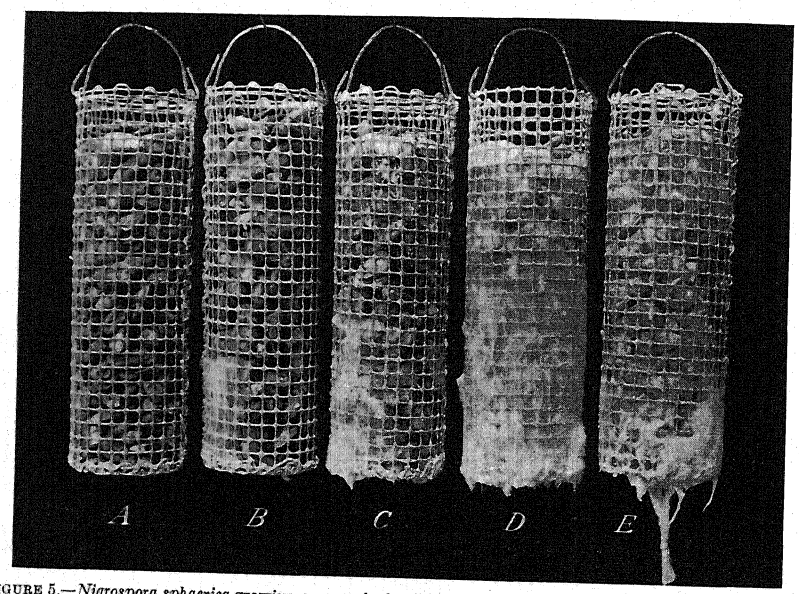


FIGURE 5.—*Nigrospora sphaerica* growing on corn in four baskets, *B* to *E*. No growth occurred in *A*. The storage period was 3 months at 70° F. with constant moisture of the grain as follows: *A*, 20.7 percent; *B*, 22.6; *C*, 23.4; *D*, 26.1; *E*, 29.2. At the same moisture content *Gibberella zeae* and *Diplodia zeae* present a similar appearance except that kernels infected by *G. zeae* turn a reddish color and portions of the mycelium may show yellowish to reddish hues, and those infected by *D. zeae*, which is a white mold, turn a brownish color and the mold usually is denser than that shown.

theless of very common occurrence. It is rarely found when plating surface-sterilized rot-damaged grain but is often observed when plating apparently sound grain. The explanation seems to be that *Cephalosporium* is not ordinarily a rot-producing organism. This belief was further strengthened by the storage experiments here reported. As is true with *F. moniliforme*, much of the *C. acremonium* infection is superficial enough so that thorough surface disinfection frees half or more of the kernels carrying this fungus. Nevertheless, by careful selection of corn ears, grain composites were prepared carrying 30 or more percent of *C. acremonium*, and no other fungus, after surface disinfection.

When corn grain was placed in the storage equipment without surface sterilization, other fungi developed in profusion at the higher moistures and *Cephalosporium* could not be detected by microscopic examination up to 27 percent moisture of the grain, which was the highest used. With surface-sterilized grain a pure growth of *Cephalo-*

sporium developed at suitable moistures. At 23.4 percent typical spore heads developed on sparse mycelium at the tip ends of the kernels and some of the kernels developed narrow longitudinal white streaks which were caused by the mycelium lifting the colorless seed coat away from the yellow endosperm. With higher moistures *Cephalosporium* growth became more abundant, but even at 27 percent moisture the growth was limited to the region at the tip ends of the kernels and there were no prominent indications of rotting after 3 months' storage.

DISCUSSION

A considerable number of tests were made with natural shelled corn, that is, unsterilized and uninoculated. Members of the *Aspergillus glaucus* group never failed to appear at 14.5 to 15.5 percent moisture in the grain and sometimes without apparent competition from other organisms. Some other aspergilli appeared at slightly higher percentages of moisture and some penicillia regularly appeared near 17 to 18 percent. They could be readily identified as penicillia among the aspergilli by the use of a microscope. A vertical illuminator which throws the light down on the object through condensers built around the objectives was found especially useful for distinguishing the various kinds of fungi. *A. niger*, a very easily recognized fungus, appeared in only about two-thirds of the tests at moistures suitable for its growth. Either this fungus was not universally present or slight amounts of it might have been inhibited by the growth of other organisms. This fungus, unlike some others, is so conspicuous that it would hardly be overlooked if present. *A. flavus* appeared in less than one-third of the tests.

In interpreting the data it must be borne in mind that in each test only a limited number of moisture constants were used with intervals of ½ to 2 percent of grain moisture ranging between them. Thus the exact moisture limit for fungus growth could not always be observed in any one experiment. Furthermore, experimental results did not always check closely. For instance, *Aspergillus wentii* was observed growing naturally on corn in two different tests made in two different seasons. In one case it was found at 15.1 percent but not at 14.3; in the other it was found at 16.2 but not at 15.2. Therefore, the data in table 3 show it as not present at 15.2 in one test, but as present at 15.1 in another. This fungus was not observed in 19 other tests with unsterilized seed, but this does not necessarily mean that it was absent, for it is not particularly conspicuous and can easily be overlooked when other fungi are abundant.

The variations in the results obtained in different tests can probably be accounted for by the fact that the corn used at different times came from different lots grown under different conditions. Moreover, different strains of the fungus species were present at different times. The results obtained with *Fusarium moniliforme* were especially variable, and this was charged primarily to the fungus itself as explained earlier under the discussion of that fungus.

Except in seed lots that had been carefully selected for freedom from *Fusarium moniliforme*, this fungus predominated above a moisture content of 23 percent provided *Diplodia zeae* was not present and provided the desired moisture was added at one time or the corn had not previously been dried. If dry corn was hung over solutions and the mois-

ture was taken up from the atmosphere the rise in moisture was slow (fig. 2) and aspergilli made heavy growth before *F. moniliforme* could get started. A fungus already in possession of the field may block the growth of another fungus which might have predominated if it had had an equal opportunity.

At a moisture suitable for vigorous growth of several fungi, the relative amount of inoculum of each kind present often determines which one will predominate. When different lots of unsterilized corn were dusted with several pure cultures of aspergilli and penicillia, respectively, the result in a number of cases was a growth of an almost pure culture of the fungus used for inoculation, almost as pure as though pure culture methods had been used.

When a *Penicillium* or *Aspergillus* species was found growing on grain at its lowest moisture limit it not only made weak growth but it also grew only on a minority of the kernels. At a slightly higher moisture the growth was not only more vigorous but it also occurred on a higher percentage of kernels. This appeared to be a combined effect of chance inoculation and actual difference in moisture content of individual kernels. It is also probable that some kernels possess resistance of a chemical nature to some extent (*11*). When kernels were thoroughly dusted with spores of one of these fungi before the experiment started, the fungus occurred on a much higher percentage of kernels even though it made weak growth for lack of water; still some kernels remained free from fungus growth. It has already been shown (table 2) that different kinds of corn may take up moisture at different rates or may come to equilibrium with their surrounding atmosphere with different percentages of moisture in the grain. This being true, individual kernels, especially of an open-pollinated variety, will no doubt behave differently in this respect also.

Actual damage to grain from rot was never observed at the lowest moisture limit for fungus growth. Damaged kernels of the kind classified as "commercial damage" by grain inspectors usually occurred to some extent with the various fungi tested at a moisture of about 1½ to 2 percent higher than the minimum moisture for growth. If the storage period had been longer, the critical moisture for development of damaged kernels might have been slightly lower. With temperatures higher than 70° F. the minimum moisture requirement and the margin for commercial damage both appear to be lowered slightly. When temperatures become sufficiently high heat damage takes place in addition to rot damage.

The oxygen requirements of the corn-rot fungi studied were very moderate. When 100 g of corn was suspended over a salt solution in a sealed bottle containing nearly 2 liters of air, growth with the various molds usually proceeded nearly as well as in an aerated bottle. A sour alcoholic odor developed in sealed bottles in which the moisture of the corn was over 16 percent. This odor was not observed in bottles aerated with only a fine caliber glass tube as mentioned in the discussion of methods. Rapid aeration with sterile air was used to carry off the chlorine when the corn had been surface-sterilized with chlorinated lime. If the sealed bottles had been filled full with grain, lack of aeration might have greatly retarded mold growth as Swanson found with wheat (*18*). In deep storage bins aeration also is exceedingly limited. Duvel (*5*) made observations in a 65-foot elevator bin filled with shelled corn at 17.0 to 18.8 percent moisture.

Storage was carried out during the late winter and spring months, the temperature being 36° to 39° F. when the experiment was started. In 5 weeks' time the mustiness had extended down to 7 feet below the surface but no lower. In 7 weeks no mustiness was observed at a 12-foot depth although there was an abundance of molds at the surface. When the bin was emptied after 9 weeks' storage the lower half still appeared to be free from significant mold growth.

SUMMARY

Shelled yellow dent corn was stored in atmospheres maintained at constant humidities of various degrees by placing it in wire baskets over salt solutions in closed containers. Storage was for a period of 3 months at a temperature of 70° F. Three methods of aeration were tried. The one supplying the least oxygen appeared adequate for growth of the fungi studied, but more liberal aeration was supplied for most of the tests.

Growth limits of a number of fungi as determined by moisture in grain was studied in two ways, in competition with surface-borne fungi, and in pure culture after surface-sterilizing the grain. The fungus to be studied was supplied by either selecting corn carrying the fungus as an internal infection or by inoculating the grain.

Different strains of corn showed distinct differences in the percentage of moisture in the grain when in equilibrium with a saturated atmosphere. There was good evidence also for believing that individual kernels showed variations in moisture content in experiments with open-pollinated corn. Thus one would not be able to predict accurately the moisture content of grain stored in an atmosphere with a known constant humidity.

Aspergillus glaucus grew at 14.3 percent moisture of grain, which was lower than could be utilized by any other fungus, but several other aspergilli appeared at only slightly higher moistures. *A. flavus* and *A. niger*, however, were not found below a moisture content of 18.3 percent.

Five species of penicillia were found to vary in their minimum moisture requirement from 15.6 to 20.8 percent. The blue color of the germ known to grain inspectors as "blue eye" was found to be caused by the growth of certain kinds of penicillia between the germ and the seed coat. *P. notatum* caused blue eye at a minimum moisture of 16.7 percent, while *P. palitans* required 19.5 percent, in these experiments. In each case somewhat more moisture was required for the extensive development of the blue-eye condition.

The minimum moisture requirement for *Fusarium moniliforme* ranged from 18.4 to 21.2 percent in tests in which competition from other organisms was excluded. A similar range was observed where competition was allowed. This was a wider variation than was observed with any other fungus species and demonstrated a significant variation in the moisture requirement of different strains of *F. moniliforme*.

Various aspergilli and penicillia grew well in mixed combinations in natural uninoculated seed. When the spores of a single species of these same fungi were dusted on the grain before a storage period was started, that particular fungus predominated more or less to the exclusion of all others provided moisture conditions were suitable.

Above 23 percent moisture *Fusarium moniliforme* competed well and often predominated over all other fungi except when *Diplodia zeae* was present. While in many cases one fungus tended to dominate another under growing conditions suitable for both, the relative abundance of inoculum of each one present was often an important factor in determining dominance.

Diplodia zeae, *Gibberella zeae*, and *Nigrospora sphaerica* all grew well on corn above 21.5 to 23 percent moisture content in the absence of competition from other organisms. When forced to compete, *D. zeae* was the most aggressive and *G. zeae* next. *N. sphaerica* was very weak.

For the production of commercial damage to the grain from rot by fungus growth an increase of 1½ to 2 percent in moisture over the minimum moisture requirement for growth was usually needed under the conditions of these experiments.

While *Cephalosporium acremonium* grew well on naturally infected corn under pure culture conditions, at moistures of 23.4 to 27 percent, it was not observed to cause commercial damage.

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SOME CORRELATIONS IN PLANT-TISSUE COMPOSITION, DECOMPOSITION PRODUCTS, AND EFFECT UPON CROP ROTATION WITH TOBACCO[1]

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INTRODUCTION

The concept of crop rotation has been developed largely through long experience with increased or decreased yields. In some degree the ideas on crop rotation have become a matter of tradition with little or no thought given to the reasons why one rotation is a success and another a failure. The underlying causes have long been suspected to be the action of residues of one crop upon the succeeding crop. The chemical and physical character of the soil is also undoubtedly affected by the selective nature of different plant membranes as receptors of ions and the element of time in the decomposition of residues.

Tobacco plants are especially sensitive to certain rotations. In farm practice, tobacco reacts most favorably when preceded by tobacco. Tobacco planted in rotation with ragweed and horseweed does not suffer any appreciable harm, but rotation with timothy or corn—in fact, with any of the grasses except redtop—has been found during certain seasons to be extremely unprofitable. Tobacco following corn, timothy, or clover is likely to suffer severely from brown root rot. In rotation with redtop, however, it seems to be less severely affected, but the trouble is not as perfectly eliminated as when either of the two weeds, ragweed or horseweed, is used, or when tobacco follows tobacco.

Examination of 45 different plants to learn the relative rate of decomposition of their tissues and the subsequent rate of ammonification and nitrification indicated that of all the seed plants used redtop decomposed most slowly. Then for this work followed timothy, corn, horseweed, and ragweed. Tobacco decomposed more rapidly than any of the other seed plants.

Related to this rate of decomposition is, of course, the carbon content of the soil and its reducing capacity. In two adjacent plots of the same soil types, the carbon content was higher in the one where corn preceded tobacco than in the other where tobacco grew for 2 consecutive years. The soil's greatest reducing capacity was well indicated by more nearly complete reduction of potassium bromate.

A field plot on which corn had been grown for 4 successive years was sterilized by passing an electric current through the soil to a depth of 6 inches. Tobacco planted in this soil developed without a trace of brown root rot. Plots on which corn had grown for 4 years were planted to *Chenopodium album* and *Amaranthus retroflexus* for 1 year. Tobacco grown on these plots the next year was much less affected by root rot than tobacco on a plot where corn had been grown for 5 years. Chemicals containing phenol as orthocresol, paracresol, and

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metacresol also improved the soil for tobacco both in pots and in the field. Another method that helped to prevent the rot was artificial drying of the soil previous to planting.

It seems that an organism, or a class of organisms, is specific for types of organic decomposition and that the products of their decomposition are different. Whether it is the activity of facultative parasites, obligate parasites, or saphrophytes, or their products which are harmful has not been discovered.

In the present work it was the author's desire to correlate the results obtained in the field with certain chemical structures and decomposition products arising from microbiological processes.

REVIEW OF LITERATURE

Differences in crop yield incident to different systems of rotation have been ascribed in part (1) to the toxic products arising from decomposition of specific types of plant tissues, and (2) to the ability of certain plants to remove from the soil some elements in too great abundance and thus leave to other plants little on which to subsist. The toxic compounds have been found to be relatively labile and readily changed by oxidation. Many of them contain an aldehyde group, either aliphatic or aromatic, or an unsaturated linking in their structure. Oxidation involves the conception of change of valence—loss of electrons. Schreiner and Shorey,[2] Breazeale,[3] and Collison [4] are of the opinion that these abnormal compounds influence the subsequent crop.

Experiments by Doran [5] and Eisenmenger [6] indicate that the infusions of certain plants cause retardation of growth of tobacco when applied to soil, and that the nitrogen fractions of different plant infusions are different. Nitrogen of tobacco attains an end point of ammonia or nitrate more rapidly than does the nitrogen of corn stover.

METHODS

The plants used for this experiment were corn (*Zea mays* L.); timothy (*Phleum pratense* L.); redtop (*Agrostis alba* L.); tobacco (*Nicotiana tabacum* L.); horseweed (*Erigeron canadensis* L.); and ragweed (*Ambrosia artemisiifolia* L.).

Each plant was dried, ground, and placed with soil in crocks of 1-gallon capacity. The total nitrogen for each plant species was determined, as was also the nitrogen of the soil. To each crock was added a different type of the ground plant tissue in such amount as to provide 11.09 g of nitrogen. This means, of course, that the amount of total organic matter was not the same in each crock. The crocks were kept in the greenhouse at approximately the same moisture content for 7 weeks. They were started at different times in order to permit the analyses to be done in sequence.

[2] SCHREINER, O., and SHOREY, E. C. THE ISOLATION OF HARMFUL ORGANIC SUBSTANCES IN SOILS. U. S. Bur. Soils Bull. 53, 53 pp., illus. 1909. See pp. 15–16.

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Plants at the usual stage of harvest maturity were chosen for the work. The ragweed and horseweed had ceased flowering and the lower leaves were dry.

At the end of 7 weeks each crock was removed, the material shaken with distilled water and filtered, first through coarse cloth and then through a Chamberland-Pasteur filter. A portion of the soil was dried at 110° C. and the moisture determined. The total nitrogen content of the soil was determined in this phase of the work by the regular Kjeldahl method.

In determining the total nitrogen of the extract, an aliquot portion, 50 cc, was made acid with sulphuric acid containing salicylic acid and taken down to dryness on a water bath. The modified method of Ranker was then followed.

For ammonia nitrogen, 100 cc of the soil extract was made alkaline with calcium oxide and the ammonia distilled into a standard acid (0.02 N) by steam distillation.

Protein nitrogen was determined by precipitation with ferric oxide hydrosol while hot, and filtered. The nitrogen in the residue was determined by the regular Kjeldahl method of digestion and distillation.

Amides were determined as follows: From 200 cc of protein filtrate, the ammonia nitrogen was driven off by the use of steam distillation and calcium oxide. After the remaining solution had cooled, sufficient sulphuric acid was added to make it equal to a 5-percent solution. The filtrate was hydrolyzed for 2 hours by boiling, a reflux condenser being used. At the end of the hydrolysis period, the solution of ammonia was driven over by steam distillation into standard acid (0.02 N).

Humin nitrogen was determined by taking the residual material in the flask from which the hydrolyzed amide, or ammonia, had been distilled, filtering it while hot, and treating the residue by the Kjeldahl method used in the protein determinations.

After ammonia nitrogen had been removed from a portion of the soil filtrate, the alpha amino acid nitrogen was determined by using the Van Slyke apparatus.

The nitrate nitrogen content was found by determining the total nitrogen after reducing the nitrates in the acid solution with metallic iron and comparing this with a similar portion which had not been reduced. The difference represents the nitrogen derived from nitrates.

In a separate experiment, the carbon of each of these plant tissues was determined by an electrical combustion method.

All analyses represent triplicate trials, and most of them are the average of six trials.

RESULTS AND DISCUSSION

After the materials had been subjected to decomposing agencies, the grasses appeared to be least decomposed, in some instances not even losing their original configuration. This was especially true of redtop, which seemed to be very slow in becoming an integral part of the soil and with it assuming a homogenous mass. Tobacco, on the other hand, to all appearances decomposed most rapidly. None of the fibers maintained their original identity. This was true of horseweed and ragweed also, but to a slightly less degree.

The carbon content of the various soils at the expiration of the period allowed (7 weeks) was different, the degree of difference depending

upon the relative slowness of decomposition. It is obvious, however, that though all soils contained the same amount of nitrogen at the beginning, not all contained the same amount of carbon. It is not axiomatic that plants with a higher nitrogen content decompose more rapidly. The amount of lignin and pentosans or products ensuing with maturity exert an influence toward preservation of the included tissue. At the end of 7 weeks, the soils to which the different vegetable tissues had been added contained the following percentages of carbon: Timothy, 2.97; redtop, 5.33; corn stover, 5.21; ragweed, 2.55; tobacco, 2.56; and horseweed, 2.48.

The grasses seem to decompose more slowly than the dicotyledonous plants used. Incidentally, it may be stated that the tissue of nonvascular land plants of the lower order decompose more slowly than do dicotyledonous plants, and that the higher order of plants, the monocotyledons (expecially the Gramineae), with few exceptions decompose more slowly than do the dicotyledonous plants. This applies to herbaceous tissue. It seems probable that grasses which can tolerate the greater soil acidity are less easily decomposed than those which require a more basic medium. Also, those seed plants which can compete in a medium of rather high acidity contain relatively less calcium and more silicon.

The relative amounts of different forms of nitrogen in a specific type of plant tissue may indicate the degree to which the materials have been built up; in other words, to what stage anabolism has progressed. When the tissues are in the process of breaking down, the end products, ammonia (under anaerobic conditions) or nitrates (under aerobic conditions), in greater abundance would indicate a greater rate of retrogression. The large percentage of proteins would indicate slow nitrification by agencies involved in the process. Larger amounts of amino acids and amides would indicate not a slow decomposition rate, but slow progress in reverting to end products—ammonia and nitrates.

The stages of decomposition in part are indicated in the data shown in table 1.

TABLE 1.—*Percentages of nitrogen in various forms found in soil extracts derived from mixing different types of plant tissue with soil and allowing them to decompose*

[Results computed on dry-weight basis]

Plant	Total of soil and plant residues	Total of soil extract	Protein	Amide	Alpha-amino acid	Ammonia	Nitrate	Humin
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Timothy	0.2523	0.0222	0.0167	0	0.0032	0.0026	0	0
Redtop	.2790	.0534	.0023	.0010	.0024	.0078	.0266	.0000
Corn stover	.2545	.0445	.0238	.0000	.0046	.0093	.0009	.0028
Ragweed	.2384	.0214	.0051	.0010	.0062	.0012	.0094	.0000
Horseweed	.2008	.0117	.0042	.0007	.0042	.0006	.0029	.0010
Tobacco	.2008	.0221	.0028	.0010	.0073	.0005	.0086	.0009

The protein values of timothy and corn are relatively high, whereas that of redtop is low. This is not in accord with the appearance of the materials when removed. But it has been observed that in rotation with tobacco redtop does not exert the same deleterious effect as timothy and corn.

The total soluble nitrogen of the extracts is higher for the grasses, though the values for timothy and tobacco are not materially different. This would imply that less denitrification took place in the grass-soil media than in the other media. Also, it is possible that the more soluble forms of nitrogen were less evenly distributed in the crock than before the more soluble forms appeared.

The amides, though exceedingly low in all media, had a tendency to appear among the plants that are less harmful in a tobacco rotation, that is, tobacco, ragweed, horseweed, and redtop.

The amino acid results showed no correlation between the plants that generally are regarded as good and those generally regarded as bad in a tobacco rotation.

Nitrate nitrogen formation in the variously treated soils indicated clearly that nitrates are not so readily formed in soils containing residues of timothy and corn as in soils containing tissues of plants regarded as favorable in tobacco rotation.

In respect to nitrogen decomposition products, corn and timothy tissues decomposed in soil have some properties in common, and in other respects redtop behaves like them. All three are highest in total soluble nitrogen, timothy and corn are highest in protein nitrogen; timothy, corn, and redtop are relatively higher in amides and ammonia; and ragweed, horseweed, and tobacco are high in nitrates from soil extract. Nitrates in excess of others would suggest less reductase activity.

It would seem that corn and timothy are in one class, ragweed, horseweed, and tobacco in another; and that redtop has some characteristics of both classes.

Some other properties of plant tissues were determined in another experiment in order to extend the correlations. Table 2 indicates some of these relationships.

TABLE 2.—*Percentages of organic constituents of plants as indicating the relative degree of ammonification and nitrification of organic nitrogen during 63 days and growth-promoting capacity of plant-tissue decomposition products when 0.41 g of nitrogen from different plants was added to soil*

[The crocks containing the soil were of 8 pounds capacity]

Plant	Total nitrogen	Total carbon	C/N ratio	Pentosans	Lignin	Average weekly total ammonia and nitrate nitrogen in soil	Dry weight of barley [1]
	Percent	*Percent*		*Percent*	*Percent*	*P. p. m.*	*Grains*
Timothy	0.86	50.0	58	18.7	13.9	4	2.1
Redtop	1.02	45.9	45	27.2	9.8	2	1.4
Corn stover	.86	47.6	55	21.3	5.2	13	1.2
Ragweed	2.15	45.1	21	14.8	2.1	53	5.31
Tobacco	2.88	38.2	13	12.7	4.4	136	12.4

[1] Barley grown on soil to which 0.41 g of nitrogen from different plants was added.

Data for horseweed are not included, because no tissue of this plant was available.

Those plants that contain the lowest percentages of nitrogen, the highest percentages of carbon, have the highest carbon-nitrogen ratio,

a high percentage of pentosans, and the highest percentage of lignin, are the plants which, when placed in soil for a period of time, have the highest percentage of total soluble nitrogen, the lowest percentage of amides, and the highest percentage of ammonia nitrogen. Those plants that have the above-mentioned properties, also are characterized by low ammonification and nitrification values in soils, and low growth-promoting properties of barley (when all receive the same amount of nitrogen derived from different plants). Redtop resembles timothy and corn in all respects except in residual protein in the soils. Furthermore, the ammonia nitrates formed in the soil from redtop residues have a nitrification capacity similar to that of the plant residues not of the grass family—tobacco and ragweed.

In field practice it has been found satisfactory to follow ragweed by tobacco. On the college farm, redtop has proved more satisfactory than either corn or timothy for preceding tobacco, but never so satisfactory as tobacco preceding tobacco.

It is reasonable to suppose that, when roots and stubble of these various plants are permitted to decompose, the decomposition is slower, for the roots are invariably lower in nitrogen. It seems reasonable to suppose that high lignin and high pentosans would invite a general class of decomposing agencies different from tissues of simpler composition.

Little is known about the end products of decomposition of lignin, but the general type of methoxy compounds may arise, while starches and cellulose may, depending upon the reagents, be converted into monosaccharides, acids, and other products.

The fact that brown root rot of tobacco has been entirely eliminated from soils by sterilization with electric current, orthocresol, heating, or acetic acid and by drying, suggests that life processes are a factor in the malady, for none of these procedures would destroy the abnormal compounds associated with soil and specific plant tissue.

In field practice, we have other plants which do not lend themselves successfully to a place in tobacco rotations. Such plants as red clover and alfalfa, though not so pronounced in their harmful effects upon tobacco, have been found undesirable. These legumes when mixed with soil and allowed to decompose for several weeks convert the organic nitrogen to ammonia and nitrate nitrogen rather rapidly; alfalfa more readily than ragweed and less rapidly than tobacco. The organic nitrogen of red clover was converted into ammonia and nitrate nitrogen in soil at a rate only slightly less than that of ragweed. However, red clover and alfalfa contain a relatively high percentage of pentosans and lignin. A sample of red clover contained 15 percent of pentosans and 13 percent of lignic acid; a sample of alfalfa contained 17 percent of pentosans and 11 percent of lignic acid. These compared with 14.8 and 2.1, respectively, for ragweed and 12.7 and 4.4, respectively for tobacco, indicate at least a higher value of lignin in the two legumes.

The explanation is possibly found in the fact that the evolution of organic nitrogen to ammonia and nitrate nitrogen occurs in the initial state of decomposition, while one of the final steps in decomposition involves the conversion of lignin to organic derivatives found in soil, frequently referred to as "humus."

It is a well-known fact that organic materials containing high percentages of lignin may, when applied to soils low in nitrogen, seriously

diminish the growth of plants. Also, when the same organic matter is applied to soils too high in nitrogen, and conducive to abnormal growth and subsequent "lodging," it may exert a favorable influence. This influence is temporary, for when lignin is once decomposed, the result is more favorable than if none had been added to decompose. It has been suggested by observers that an old timothy sod is not as deleterious to a subsequent tobacco crop as one that has grown for only a few years. The inference is that the residues of previous years have to some degree overcome the results of newer decomposition activities.

Plant tissues in the early stages of development contain an appreciable amount of lignin, but when the plants are plowed under for green manures, the deleterious effects are not apparent. At this stage of growth, plants contain an abundant quantity of bound water and the rapidity of decomposition is augmented.

It would seem that what is apparently an unfavorable rotation for tobacco may be unfavorable for other crops, but to a less degree, for the tobacco plant is more sensitive than most plants to its environment.

Tissues of plants containing high percentages of lignin maintain their original structure, when killed, for a longer period than do those having a low content of lignin. Thus the stems and leaves of grasses and sedges have much the same configuration after winter has passed as when they were green, while tobacco, potato, and ragweed have little left to suggest their original appearance. In a general way we should be able to determine after a winter has passed those plants that would fit into a rotation system with tobacco.

SUMMARY

An attempt was made to find correlation in results relative to tobacco rotations obtained in the greenhouse, the laboratory, and the field.

From the laboratory test, an estimation was made of nitrogen fractions resulting from decomposition of different types of plant tissue in soil—corn, timothy, redtop, ragweed, horseweed, and tobacco. Also, the amounts of lignin, pentosans, carbon, and nitrogen and their ratio, rates of ammonification and nitration, and growth-promoting values were calculated when equal amounts of nitrogen from each of the different plants were added to soil.

Timothy and corn, which have been found unsatisfactory in tobacco rotation, showed a high percentage of total nigrogen, a high percentage of total soluble nitrogen, the highest percentage of protein, a low percentage of amides, a high percentage of ammonia, and the lowest percentage of nitrate nitrogen. Ragweed, horseweed, and tobacco showed lower percentages of each of these fractions except nitrates. The nitrates in this group were higher.

Redtop resembled the other grasses in the decomposition of organic nitrogen except protein and nitrates. It was relatively high in nitrates and low in protein.

The plants could be divided into two distinct groups. The first group, which comprised the grasses corn, timothy, and redtop, contained higher percentages of carbon, low percentages of nitrogen, a high carbon-nitrogen ratio, high pentosans, high lignic acid, low ammonification and nitrification, and low growth-promoting values.

The other group—ragweed and tobacco—showed lower carbon values, higher nitrogen values, a lower carbon-nitrogen ratio, lower pentosans, lower lignic acid, higher ammonification and nitrification, and higher growth-promoting values. In field practice, timothy and corn were found to be unsatisfactory for tobacco rotation. Ragweed, horseweed, and tobacco are desirable for rotation. Redtop is less desirable than tobacco and ragweed, but more desirable than timothy or corn.

It would seem that plants containing the higher values of lignin, pentosans, high carbon-nitrogen ratios, and a subsequent low tendency to protein decomposition in soil may be suspected of being undesirable for tobacco rotation.

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TWO FUNGI CAUSING LEAF SPOT OF PEANUT [1]

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INTRODUCTION

The commonly cultivated varieties of peanut (*Arachis hypogaea* L.), especially the Spanish varieties, are seriously defoliated during the summer and early fall by two *Cercospora* leaf spots. Both leaf spots occur regularly on peanuts throughout the peanut-growing areas of the United States and apparently are destructive wherever peanuts are grown (*7*),[3] often making the crop unfit for hay and undoubtedly bringing about marked decreases in yield of pods.

Although several different species of fungi have been described from various parts of the world as causing leaf spots of peanuts, Woodroof (*7*) has shown by an extensive review of the literature and a study of many exsiccati that *Cercospora arachidicola* Hori and *C. personata* (B. and C.) Ell. and Ev. are the only valid species that have been described. She has likewise shown that both fungi cause destructive leaf spotting of peanuts in Georgia, whereas these diseases in the United States were formerly attributed to *C. personata* alone.

The present work is a report of the results of an investigation of the life history and cytology of the two peanut leaf spot fungi which the writer has had under way since 1934.

MYCOSPHAERELLA ARACHIDICOLA N. SP.

THE DISEASE

While all parts of the plant are subject to attack, symptoms on the leaflets are more striking and, perhaps, more destructive. When the leaf spots are first noticeable, they appear as slightly paler areas on the upper surface of the leaflets, as though the chlorophyll was fading, or blanching, slightly. Careful observation of the lower surface at this time indicates the presence of small areas in which the epidermal cells have collapsed and lost connection with the underlying mesophyll. Following this, the lesion develops fairly rapidly, taking on a distinct yellowish hue on the upper surface of the leaflet. Often a very small necrotic area becomes evident in the center of the developing lesion, before or during the time the yellow discoloration is developing. At maturity, the leaf spots appear as distinct necrotic areas, circular to irregular in outline, and often coalesce. They range in diameter from 1 mm to 1 cm or more, and are almost always surrounded by a yellow halo of varying width which blends gradually

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[2] The author wishes to acknowledge the interest and the many helpful suggestions of Dr. B. B. Higgins during the course of this investigation. He also wishes to express his thanks to M. M. Murphy and to Dr. J. L. Weimer for making the photographs contained in this paper.

[3] Reference is made by number (italic) to Literature Cited, p. 332.

into the green of the leaf on the outer edges (fig. 1, *C*, *D*). On the upper surface, the necrotic areas vary from reddish brown to black, while on the lower surface they generally appear in lighter shades of

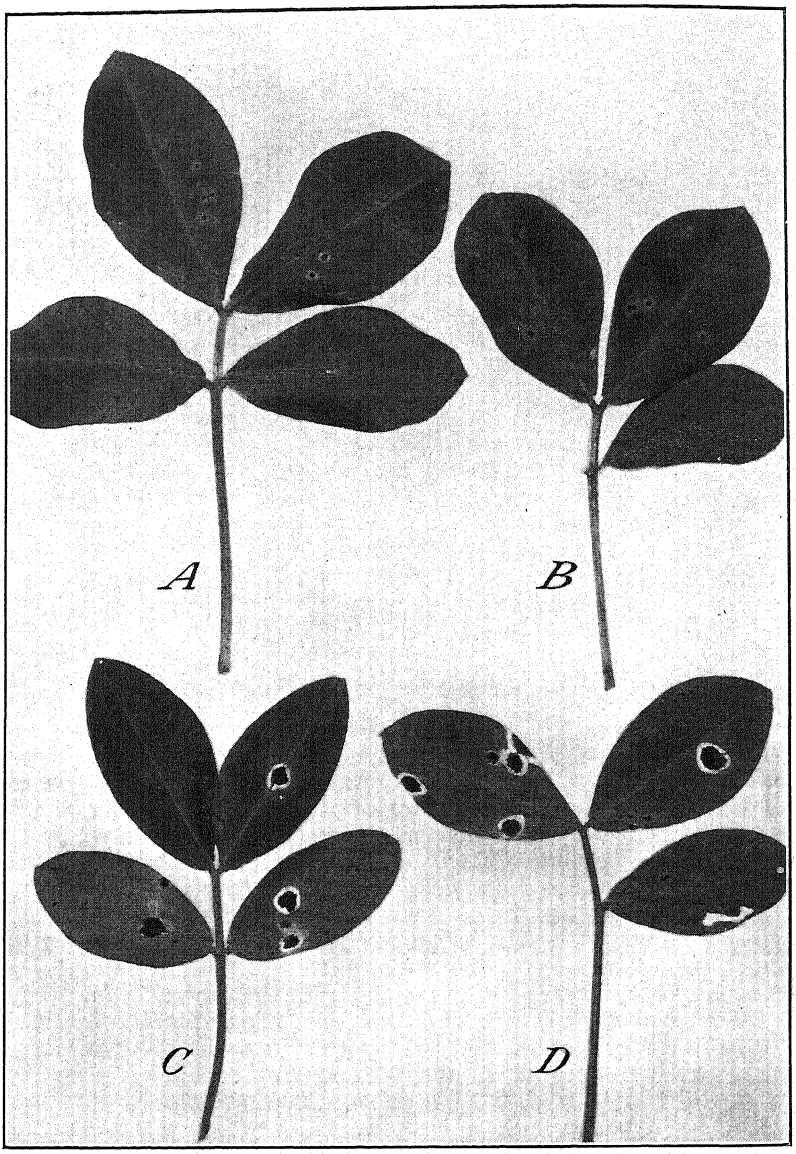


FIGURE 1.—*A* and *B*, Peanut leaflet inoculated in the greenhouse with conidia from ascospore cultures of *Mycosphaerella arachidicola*. Photograph taken 3 weeks after inoculation. Only the terminal leaflets were inoculated. *C* and *D*, Typical lesions of *M. arachidicola* on peanut leaflets from field collections.

brown to tan. The halos are much less distinct or even indistinguishable on the lower surface. Observations tend to show that the halo surrounding each lesion is perhaps in some way related to the carbo-

hydrate content of the leaf cells, since they appear best developed on leaves supposedly high in carbohydrates.

Evidence secured from many conidial inoculations indicates that infection is accomplished through either leaf surface, though the inoculum adheres best on the lower surface, and that penetration occurs directly through the lateral faces of the epidermal cells or by way of open stomata (fig. 4, *A*). Sections of young lesions show the mycelium of the parasite to be first intercellular, but the host cells are killed rapidly somewhat in advance of the mycelium, and the mycelium then penetrates the walls of the dead cells. No evidence of haustoria could be found. Conidiophores are at first developed only on the upper surface of the lesions, but often become amphigenous with age. They develop from subepidermal or subcuticular hyphae, which usually continue growth and produce more or less definite stromatic bases. The conidiophores quickly become yellowish brown and as conidia are formed and abjointed apically, the conidiophores continue to grow past them, and thus become geniculate. The conidia are usually attached at the broad end and leave definite scars on the conidiophores as they are abjointed and fall away.

The conidia are colorless to pale olivaceous in color, obclavate, often considerably curved, and measure from 37μ–$108\mu \times 2.7\mu$–5.4μ. The length, and particularly the number of septations, are influenced by weather conditions at the time of conidial production. The number of septations varies from 3 to 12; shorter spores and fewer septations being associated with dry weather.

Conidia have been observed to germinate within 3 to 8 hours when moisture, oxygen, and temperature conditions were ideal. Germ tubes emerge from the terminal cell at either or both ends of the spore, and often from other cells as well. When completely covered by water, so that the oxygen supply is diminished, the spores rarely germinate at all, but often the cells become distorted through swelling. This condition is likewise evident when insufficient moisture is present. Abundant germination was always secured when the spores were placed on the surface of 2-percent agar at room temperature.

SPERMOGONIA

Spermogonia have been reported for this fungus on several occasions, both from field collections (*6*, *7*) and from studies on herbarium material. No record appears in the literature, however, of any studies on their development and structure. Spermogonia begin to appear on fallen leaflets at Experiment, Ga., during the latter part of September or early in October and continue to develop throughout the fall and winter. Occasionally young spermogonia are found as late in the spring as March, but this is rare. They are developed on either leaf surface, but are more often epiphyllous. They originate either within old conidial stromata or within separate stromata that develop after the death of the leaflets.

It is difficult to identify the earlier phases of spermogonial development, but in those cases that are certain the fundaments arise either subcuticularly or subepidermally, by the continued branching and interlacing of hyphae, which soon form compact globose to somewhat oval-shaped masses of stroma that continue to become larger and more complex until the cuticle or epidermis is ruptured (pl. 1, *A*).

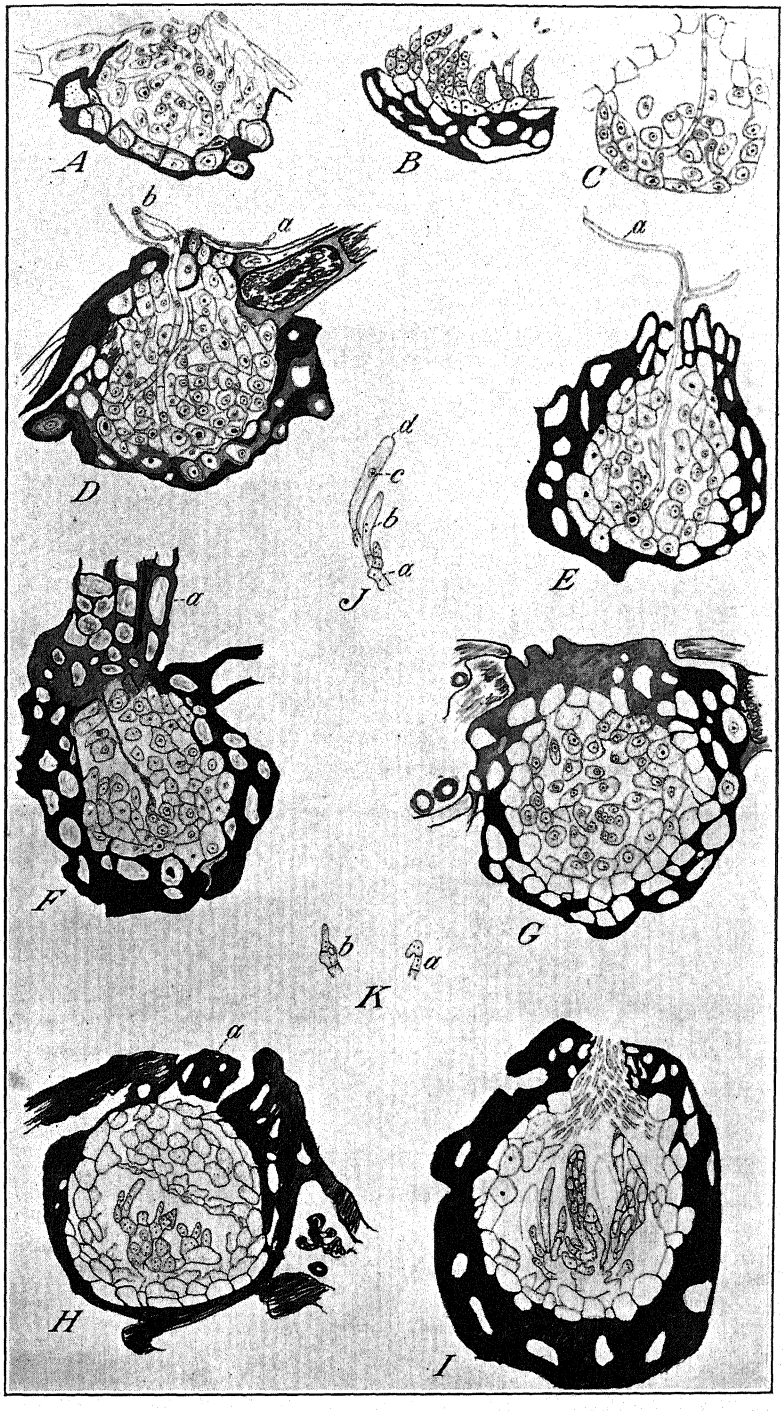
As development progresses, cells toward the center of the young spermogonium enlarge, the nucleus of each cell divides twice, and ultimately four uninucleate spermatia are formed in each cell after the fashion first described by Higgins (*3, pt. I*) and later by Jenkins (*5*) (pl. 1, *B*). The spermatia usually lie in tetrads but often appear in linear arrangement, and are liberated one at a time through a sterigma on each spermatial mother cell. After they have discharged their spermatia, the mother cells disintegrate and in so doing create enough bulk and pressure to force the spermatia out of the spermogonium. At maturity, the cavity is filled with spermatia and disrupted mother cells, surrounded by a single layer of spermatiferous cells, which is in turn enclosed by the spermogonial wall. Mature spermatia are rod-shaped, 1.5μ–$3\mu \times 0.5\mu$–1μ, and each possesses a deeply staining, centrally located, nucleus. Spermogonia which develop within conidiophore bases seem to have a different initial history. The pseudoparenchymatous cells toward the center of the stromatic mass are influenced to renewed activity, as evidenced by their staining reactions, and the cells lose their pseudoparenchymatous relationship. From this point onward, development is identical with the above-described process.

PERITHECIA

During early development, perithecia are practically identical with spermogonia as regards time, location in the leaf tissue, and mode of origin. They differ markedly, however, in that the cells toward the center of the young perithecium do not become enlarged as in the young spermogonium. Rather early in the development of the perithecium, one to several deeply staining archicarps, each with a somewhat enlarged, uninucleate basal cell and a long, several-celled trichogyne, are produced (pl. 1, *C, D*). The nuclei of the basal cells are very prominent, while those of the trichogynes are somewhat smaller and stain somewhat less distinctly. It is extremely difficult to identify the very young archicarps, but in those cases in which the writer could be certain, they appeared to arise as specialized branches from the plectenchymatous hyphae at or near the base of the young perithecium. The trichogynes often weave intricately among the sterile hyphae of the young perithecium and emerge to coil (pl. 1, *D*) or ramify (pl. 1, *E*) along the surface of the leaf. In certain preparations (pl. 1, *E*) the trichogyne is not only branched, but is apparently encased by a (chitinous?) sheath, except at the tip. This feature, if constant, may help to explain why the trichogynes are receptive only at the tips. The fact that the trichogynes often branch, an observation, which to the writer's knowledge, has not heretofore been reported

EXPLANATORY LEGEND FOR PLATE 1

A, Young spermogonium of *Mycosphaerella arachidicola* in the upper surface of a leaflet, showing the enlargement of cells near the center; *B*, Portion of a mature spermogonium showing origin of spermatia; *C*, Young perithecium showing origin of the archicarp; *D*, Young perithecium containing two archicarps (note the attached, empty spermatium (*a*) and the sperm nucleus within the coiled trichogyne (*b*)); *E*, Young perithecium with a branched trichogyne. The exposed parts are covered by a (chitinous?) layer except at the tip (*a*); the sperm nucleus has reached the base of the trichogyne; and the protoplasm of the trichogyne is disorganized; *F*, Young perithecium developing in the base of a conidiophore fascicle (*a*), showing the sperm nucleus within the basal cell of the archicarp; *G*, An older perithecium. The ascogonium contains four pairs of nuclei; *H*, Early development of ascogenous hyphae. The paired nuclei within the branched ascogonium are embedded in dense masses of cytoplasm. The stromatic plug (*a*) is being pushed out in the formation of the ostiolum: *I*, Mature perithecium, showing ascogenous hyphae, young asci, mature asci, and periphyses; *J*, Ascogenous hypha (*a*) young ascus prior to fusion of nuclei (*b*): young ascus with the primary ascus nucleus (*c*); and a hyaline area at the apex of the ascus (*d*); *K*, beginning of crozier formation (*a*), and the process completed (*b*). All sketches drawn to scale with the aid of a camera lucida. × 1,320.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

in this genus of fungi, raises interesting speculations as to phylogenetic relationships.

SPERMATIZATION

Spermatia become attached to the tip or very near the tip of the trichogyne. Higgins (*3, pt. III*) was able to distinguish a receptive papilla on the trichogyne in *Mycosphaerella tulipiferae*. Though this feature could not be distinguished in the present work, its presence is suspected. The actual passage of the sperm nucleus from the spermatium into the trichogyne was not observed. Observations on many preparations, some showing attached, nucleated spermatia, and others showing attached, empty spermatia, together with the fact that attached, empty spermatia are consistently correlated with the presence of an extra, readily distinguishable nucleus in the trichogyne, have convinced the writer that spermatization does occur regularly (pl. 1, *D, E*). It is significant to note, also, as first described by Higgins (*3, pt. III*), that the nucleus and cytoplasm of the trichogyne disintegrate progressively as the sperm nucleus migrates down the trichogyne. In the present work, the presence of a multicellular archicarp makes this process all the more convincing (pl. 1, *C, D, E*). The writer believes that a more critical study of this phase of development would demonstrate the actual passage of the sperm nucleus into the trichogyne. The sperm nucleus gradually enlarges as it migrates down the trichogyne toward the nucleus in the basal cell. Shortly after it reaches the basal cell, it is indistinguishable from the basal nucleus. No fusion takes place between the sperm and basal nuclei. Instead, the ascogonium enlarges, and as it does so, the nuclei divide conjugately.

MATURATION

Several to many pairs of nuclei are formed in the enlarged ascogonium before ascogenous hyphae become evident (pl. 1, *G*). Shortly before and during the time ascogenous hyphae are being formed, the enlarging ascogonium becomes variously branched and contorted (pl. 1, *H*). At this time denser masses of cytoplasm enclose the paired nuclei (pl. 1, *H*), in this respect resembling the situation found in *Mycosphaerella tulipiferae* (*3, pt. III*). Initial ascogenous hyphae (pl. 1, *H, I*) are scarcely distinguishable from ascogonial branches, but later, when crozier formation begins (pl. 1, *K*), those hyphae that will form asci are readily distinguishable. Crozier formation differs in no important respects from that described by Higgins (*3, pt. III*). As the young ascus begins to elongate, the paired nuclei fuse to form the primary ascus nucleus. This nucleus enlarges somewhat (pl. 1, *J, c*) and begins a series of three divisions, resulting in the production of eight nuclei. Single-celled spores are formed by the condensation of cytoplasm about each nucleus. Soon the nucleus of each young spore divides once, and cross septa are formed which divide each spore into two-celled, biseriately arranged spores, the upper cell usually being somewhat thicker and shorter than the lower. At the same time, the ascogenous hyphae directly beneath and the thin-walled parenchyma cells above the developing asci are crushed by the pressure of the elongating asci (pl. 1, *H*).

During ascospore formation, the cytoplasm remains uniformly more hyaline at the apex of the ascus. It is in this area that the bitunicate nature of the ascus first becomes distinguishable (pl. 1, *I*, and *J, d*).

Not all the asci of a given perithecium mature at the same time. Almost without exception, practically all phases of ascus development were found in perithecia which were actively discharging mature spores (pl. 1, *I*).

As the first ascospores begin to mature, certain cells near the apex of the perithecium begin to multiply, and as this area is cracked open by the stress of unequal growth pressure (pl. 1, *H*, *a*) these cells line the ostiolum with periphyses (pl. 1, *I*). The periphyses are destroyed by the asci as they elongate and push through the ostiolum during spore discharge.

Several factors influence perithecial formation in nature. One of the limiting factors at Experiment seems to be sufficient rainfall during spermogonial discharges. During the several seasons the writer has pursued this study, it has been shown conclusively that unless the overwintering leaflets are wet during the period in which spermatia are being discharged, no perithecia are formed. The experiment was outlined in three parts, one, in which the leaves were sheltered and kept dry, another, in which the leaves were dependent on rains and dew for spermatization, and another in which the leaves were artificially sprinkled. Perithecia were produced only when the leaves were artificially sprinkled or when rain fell during spermatial discharge. Even under ideal conditions, perithecia are none too abundant, which may be explained in part by the fact that the leaflets, except for the lesions, disintegrate rapidly, thus affecting the nutrition of the overwintering fungus.

Temperature likewise seems to play an important part in perithecial production. Overwintering leaves were brought into the laboratory during February and March and placed in moist chambers. In each instance, mature perithecia and spores were secured in from 2 to 3 weeks, while mature perithecia and spores were never found in the field earlier than May 31. Spore discharge continued over a period of several weeks.

DEVELOPMENT IN CULTURE

Conidia germinate readily on agar under favorable conditions. Single- and multiple-spore isolations of conidia were obtained by streaking water suspensions of spores on tap-water agar in Petri dishes and transferring single or several spores to various types of agar in tubes. Satisfactory cultures were obtained on several agar media as malt, onion, synthetic, potato-dextrose, whole-milk, rice, oatmeal, corn-meal, peanut-leaf decoction, and on such other media as surface- and steam-sterilized peanut leaves and stems. On all these substrates, growth from single spores is visible to the unaided eye in about 3 days as whitish, sharply delimited, pulvinate colonies. Conidia are produced in each case in from 56 to 72 hours at room temperature (fig. 2, *C*). After 4 to 7 days cultures on all media assume an olivaceous color, and except in onion agar, on which conidial production is sustained for several days, conidial production ceases as the colonies begin to change color. Hard agars tend to keep the cultures from spreading. After 3 to 4 weeks the cultures on all these media have spread considerably and have begun to produce spermogonia in abundance. All attempts to produce the perfect stage in culture have failed. With the advent of spermogonia, or shortly before, the colonies become almost black and very stromatic.

Failure to obtain perithecia in culture was at first attributed to lack of free water and consequent failure of spermatization, but even cultures flooded with water and others flooded with suspensions of spermatia from different isolates, failed to produce perithecia, as did others grown in liquid cultures. Older cultures were fixed and sectioned in paraffin, but no evidence of archicarps could be found.

Isolations of ascospores were obtained by placing perithecial material below inverted tap-water agar plates and allowing the ascospores to shoot and stick to the surface of the agar. In this way patterns of eight spores, representing the content of individual asci, could be obtained absolutely free from contamination. Close or distant spacing of spores was obtained readily by raising or lowering the agar plates and, consequently, single- or multiple-spore isolations could be obtained at will.

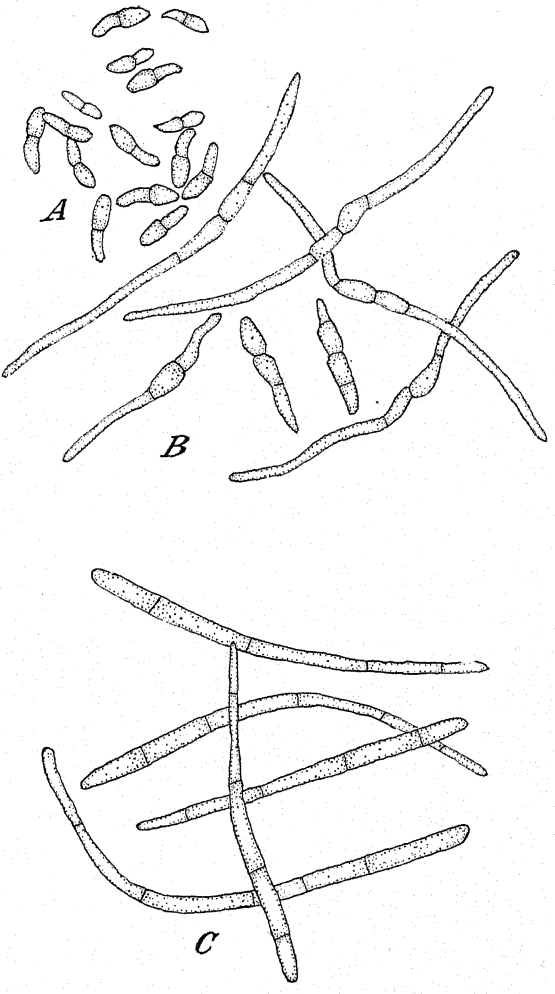


FIGURE 2.—*A*, Ascospores of *Mycosphaerella arachidicola; B*, germinating ascospores after 15 hours in tap water; *C*, Conidia from ascospore cultures. All × 750.

Ascospores germinate in from 3 to 4 hours under ideal conditions (fig. 2, *A*, *B*), but the resultant growth is scarcely visible to the unaided eye even after 5 to 6 days. Like the conidial cultures, ascospore cultures are whitish in color when first visible, but after 7 to 10 days, they, too, begin to assume an olivaceous color. Conidial production from ascospores in culture begins on any of the several media listed above after 5 to 6 days and, as before, is of rather short duration. In all respects, cultures from ascospores and from conidia are indistinguishable, except for the fact that ascospores produce a slower initial growth. Likewise, conidia produced on ascospore cultures (fig. 2, *C*) are indistinguishable from those produced in cultures from conidia and those produced on lesions on the peanut plant. After

about 3 weeks, both single and multiple ascospore cultures begin to produce spermogonia abundantly, but thus far all efforts to produce perithecia from ascospore cultures have failed.

GENETIC RELATION OF SPORE FORMS

Except for the fact that ascospores produced slower initial growth, cultures from conidia and from ascospores are identical in color, characteristics of growth, type of spores produced, and in all other measurable respects.

Since conidia are readily produced in artificial cultures, from both conidia and ascospores, these were used as the source of inoculum for inoculation tests, as well as conidia taken from peanut leaves. Healthy, growing peanut plants were selected for use in the inoculation tests, all of which were carried out in the greenhouse. Inoculum consisting of conidia in tap-water suspensions was placed on either upper or lower surface of leaflets, allowed to dry until barely moist, and then either wrapped with moist absorbent cotton and covered with cellophane or enclosed in bell glasses. Both methods proved successful and visible infections ranging from 50 to 100 percent were obtained in from 8 to 23 days. The checks remained free from infection. Under certain conditions of high temperature and low humidity, and when plants were low in carbohydrates, the percentage of infection was small and symptoms were slower in developing. The same was true when inoculations were made with conidia from ascospore cultures. Whether conidia from ascospore cultures, conidia from leaves, or conidia from conidial cultures were used, the symptoms produced were identical (fig. 1, *A*, *B*), the conidia borne later on the lesions were identical, and reisolations from these lesions were identical with the cultures from which the inoculum was taken.

Further evidence of genetic relation was found in numerous observations on sectioned material, in which definite hyphal connections could be demonstrated between spermogonia and perithecia, in which perithecia and spermogonia developed in conidiophore bases (pl. 1, *F*, *G*), and in which conidia were often produced the following spring on conidiophores arising from the exposed walls of spermogonia.

TAXONOMY

The form and development of the perithecia, the asci produced in fascicles, the absence of paraphyses, and the two-celled hyaline spores, are all clearly characteristic of the genus *Mycosphaerella* Johans. A careful review of the literature shows that no species of *Mycosphaerella* has heretofore been reported on peanut leaves, and since the peanut has no close relative in this country, there can be little doubt that this is an undescribed species. It is, therefore, designated *Mycosphaerella arachidicola* n. sp., with the following diagnosis:

Mycosphaerella arachidicola n. sp.

Syn.: *Cercospora arachidicola* Hori, 1917, Nishigahara Agr. Expt. Sta. Tokyo, Ann. Rept. pp. 26–27 (*4*).

Cercospora arachidis P. Henn. var. *macrospora* Maff., 1922, Riv. Patol. Veg. 12: 7–11.

Perithecia scattered, mostly along margins of lesions, amphigenous, partly embedded in host tissue, erumpent, ovate to nearly globose, 47.6μ–84μ×44.4μ–74μ, black, ostiolum slightly papillate; asci cylindrical club-shaped, short stipitate, fasciculate, 27μ–37.8μ×7μ–8.4μ, aparaphysate, bitunicate, eight-spored; spores

uniseriate to imperfectly biseriate in the ascus, bicellular, the upper cell somewhat larger, slightly curved, hyaline, 7μ–15.4μ×3μ–4μ (average 11.2μ×3.64μ).

Hab. In overwintered lesions produced by the conidial stage on leaflets of *Arachis hypogaea*, Experiment, Georgia, maturing during June and July.

Spermogonia: Scattered in and along margins of lesions produced by the conidial stage, ovate to globose, black, amphigenous but perhaps more often epiphyllous, embedded in leaf tissue but later erumpent, ostiolate, 45μ–75μ×30μ–75μ (average 62.25μ×48.5μ); spermatia small, rod-shaped, hyaline, 1.5μ–3μ×0.5μ, arising endogenously, usually in fours within spermatiferous cells and liberated through sterigma-like processes.

On recently fallen leaflets, maturing throughout period from October through February.

Conidial stage: Spots irregularly circular, often confluent, varying in size, 1 mm to several centimeters, pale to dark brown, surrounded on upper surface by distinct yellow halo, mostly on leaflets but also on stems late in season; conidiophores mostly epiphyllous, sometimes amphigenous when old, arising from a stroma, fasciculate, geniculate, yellowish brown, continuous to one to several septate, 20μ–45μ×3μ–6μ; conidia colorless to slightly olivaceous, obclavate to clavate, often curved, 35μ–108μ×2μ–5.4μ, 4 to 12 septate, length and septation influenced by dry weather.

Conidial stage parasitic on all above-ground parts of *Arachis hypogaea*, but mostly causing leaf spots.

Peritheciis sparsis, plerumque maculis marginatis, amphigenis, semi-immersis, punctiformibus, ovatis vel globosis, 47.6μ–84μ×44.4μ–74μ, nigris; ostiolis papillato praeditis; ascis cylindraceis clavatis, brevisme stipitatis, aparaphysatis, bitunicatis, octosporis, 27μ–37.8μ×7μ–8.4μ; sporidiis uniseriatis vel biseriatis, bicellularibus, cellula superiore leniter latiore, vix curvatis, hyalinis, 7μ–15.4μ×3μ–4μ, plerumque 11.2μ×3.6μ.

Hab. in foliis dejectis *Arachidis hypogeae*.

Spermogoniis autumno efformatis, sparsis, plerumque maculis marginatis, ovatis vel globosis, nigris, amphigenis plerumque epiphyllis, innato-erumpentibus, punctiformibus, 45μ–75μ×30μ–75μ, plerumque 62.25μ×48.5μ, spermatiis bacillaribus, hyalinis, 1.5μ–3μ×0.5μ–1μ.

Hab. in foliis dejectis *Arachidis hypogeae*.

Statu conidico in maculis orbicularibus v. irregularibus, confluentibus, magnis, ocraceis vel rubro-ferrugineis, ocraceis marginatis, plerumque foliis efformato; hyphis fertilibus plerumque epiphyllis, rare amphigenis, a stromate orientibus, fasciculatis, geniculatis, ochreis-ferrugineis, continuiis vel pluriseptatis, 20μ–45μ×3μ–6μ; conidiis hyalinis vel dilute olivaceis, obclavatis vel clavatis, plerumque curvulis, 35μ–108μ×2μ–5.4μ, 4–12 septatis.

Hab. in foliis et ramis vivis *Arachidis hypogeae*.

MYCOSPHAERELLA BERKELEYII, n. sp.

THE DISEASE

The disease produced by this fungus, like that produced by *Mycosphaerella arachidicola*, is evident on all parts of the plant above the soil. Late in the season it produces lesions on the stems, but it is predominantly a leaf spot disease. Early symptoms are indistinguishable from those caused by *M. arachidicola*, but very soon certain marked changes occur which make identification certain.

Perhaps the best diagnostic symptoms of *Mycosphaerella berkeleyii* leaf spot is the fact that the lesions are more circular than those of *M. arachidicola*, and the necrotic portions of the lesions, on both leaf surfaces, very early assume a very dark-brown to almost black color. At the same time, yellow halos are present on the upper surface of leaflets only on the more mature spots, this being in contrast to the very early-forming halos of *M. arachidicola*. Identification becomes unmistakable at or shortly before conidial production. At this time, the conidiophores are confined practically entirely to the lower surface of the leaflets and appear as definitely raised, dark-brown, stromatic

tufts, usually arranged concentrically and easily visible to the unaided eye (fig. 3). Later, the conidiophores may become somewhat amphigenous on old lesions, but even so the dark-brown, stromatic, concentrically arranged tufts of conidiophores could not be easily confused with the evenly distributed, olivaceous, more effuse conidiophores of *M. arachidicola*.

Inoculation tests with conidia indicate that the germ tubes of *Mycosphaerella berkeleyii* may enter either surface of the leaflet, through the lateral faces of epidermal cells or through open stomata, as do those of *M. arachidicola*. Sections of young and old lesions, however, show striking differences as regards the parasite-host relationships of the two fungi. The penetration hyphae of *M. berkeleyii* soon establish themselves intercellularly and remain so, sending variously branched to botryose haustoria into the cells of all leaf

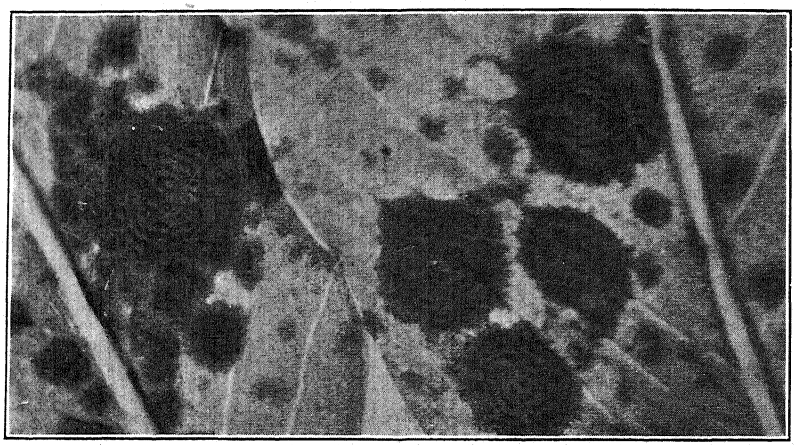


FIGURE 3.—Typical lesions of *Mycosphaerella berkeleyii*, on lower surface of peanut leaflets, from field collection. × 5.

tissues (fig. 4, *B*). The cells of the host are not killed in advance of the parasite. In fact, many preparations show haustoria established in apparently normal cells.

The conidiophores developed subcuticularly or subepidermally, and continue to become more stromatic even after they emerge through the leaf surface. At the time of conidial production, the conidiophores are coarse, reddish brown in color, except for hyaline tips, continuous to several septate, geniculate, and scarred by the abjointing conidia. The bases of the conidiophores are very stromatic, being composed of pseudoparenchyma.

The conidia are somewhat obclavate, but more generally cylindrical, except for somewhat attenuated tips, one to eight septate, pale brown to dilutely olivaceous, and measure 18μ–$60\mu \times 5\mu$–11μ. These conidia vary considerably under different conditions of moisture at the time they are formed, as do those of *Mycosphaerella arachidicola*.

SPERMOGONIA

Spermogonia have been reported for this fungus (*7*), but as in the case of *Mycosphaerella arachidicola*, no studies of their development

have been made. The cytological details of the development of the spermogonia and perithecia have not been fully worked out by the present writer, but it is hoped that this work may be completed at an early date. It was thought best to present here the work so far completed since the two organisms under consideration have been the subject of taxonomic confusion in the past, and since

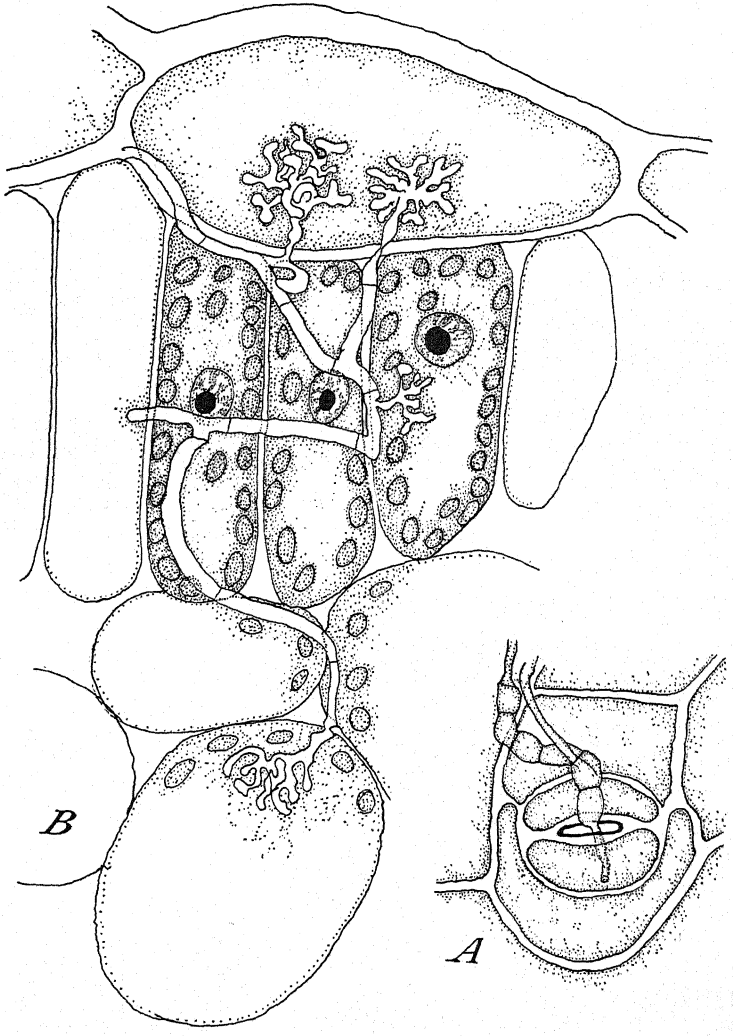


FIGURE 4.—*A*, Germ tube of conidium of *Mycosphaerella arachidicola* entering a stoma, × 750; *B*, section of a young lesion of *M. berkeleyii*, showing haustoria in epidermal and mesophyll cells, × 1320.

enough is known of their development to permit them to be definitely classified. The evidence so far obtained indicates that the spermogonia of this fungus originate and develop in a manner similar to those of *Mycosphaerella arachidicola*, and at comparable times of the year. The minor details of measurements, comparative amounts of stroma,

etc., while important, must await the completion of the cytological study.

PERITHECIA

Development of perithecia in this species appears to differ in several phases from that in *Mycosphaerella arachidicola*. Because of a greater amount of stroma in the old lesions, *M. berkeleyii* exhibits several initial variations which will require further study. Among these variations may be mentioned the fact that the archicarps are very numerous, often six to eight, in the young perithecia of *M. berkeleyii*. The large number of these structures and the fact that they interweave very intricately among other elements of the young perithecium, has slowed up this study considerably.

The work thus far indicates that spermatization is as essential to perithecial development in *Mycosphaerella berkeleyü* as in *M. arachidicola*. The same factors listed as influencing the perithecial development of *M. arachidicola* were found to influence perithecial development in *M. berkeleyii*. The experiments on the two fungi were planned and carried out concurrently. Although mature perithecia and spores were developed in moist chambers in the laboratory in March on material collected in February and March, mature perithecia and spores were not found in the field until early in July. This is an unusually late date for maturation of perithecia and spores of a species of *Mycosphaerella*, but, by allowing for a 2- to 3-week incubation period, the date of discharge of mature spores in the field is correlated very well with observations on time of initial infections on field-grown plants.

DEVELOPMENT IN CULTURE

Single and multiple spore isolations of conidia were obtained by the streak method outlined previously. In all cases, the same media were used as before mentioned. No difficulty was experienced in obtaining spore germination and conidial production in artificial culture. Given similarly ideal conditions, conidia of *Mycosphaerella berkeleyii* do not differ in percentage and mode of germination from those of *M. arachidicola*, except for the fact that the conidia of *M. berkeleyii* respond best to slightly lower temperatures.

On all substrata growth is extremely slow and becomes visible to the unaided eye only after 4 to 7 days. From the time the mycelium can be seen in culture, the hyphae are distinctly pigmented. At first the color is pale buff, but as the colonies age it becomes tawny or even reddish. Growth is always sparse. At first the aerial hyphae stand apart rather than form a compact growth as in *M. arachidicola*. Even when old, the colonies never spread much; instead, discrete tufts of tawny- to reddish-colored aerial hyphae develop from small stromatic bases. After 7 to 10 days or sometimes longer, while the colonies are still only a few millimeters in diameter, numerous spores are produced on typical conidiophores (fig. 5, *c*). In all respects, these spores are like those taken from peanut leaves, except, owing perhaps to the more constant moisture supply, they are uniformly longer. The writer tried on several occasions to cause the colonies to spread by jarring the cultures during spore production and thus knocking other spores onto the medium. In all cases colonies from these spores remained discrete from the original colony. After 3 to 4 weeks, the

colonies become stromatic, often decidedly reddish in color, and produce spermogonia in abundance. All attempts to produce the perfect stage in culture have resulted in failure; likewise no archicarps have ever been found in artificial cultures.

Ascospores isolated as described for *Mycosphaerella arachidicola* germinate in from 4 to 10 hours under laboratory conditions, but the resultant growth is scarcely visible to the unaided eye even after 7 to 8 days (fig. 5, *A*, *B*). In all respects, ascospore cultures are identical with conidial cultures, except for a slower initial growth, and produce typical conidia, after 7 to 12 days, identical in every way with those from conidial cultures. In fact, conidia are produced from ascospore cultures sometime before the colony is readily visible to the unaided eye, and the period of conidial production often extends over a period of a week or more. After 3 to 4 weeks, ascospore cultures likewise produce spermogonia in abundance, but all attempts to produce perithecia in culture have failed.

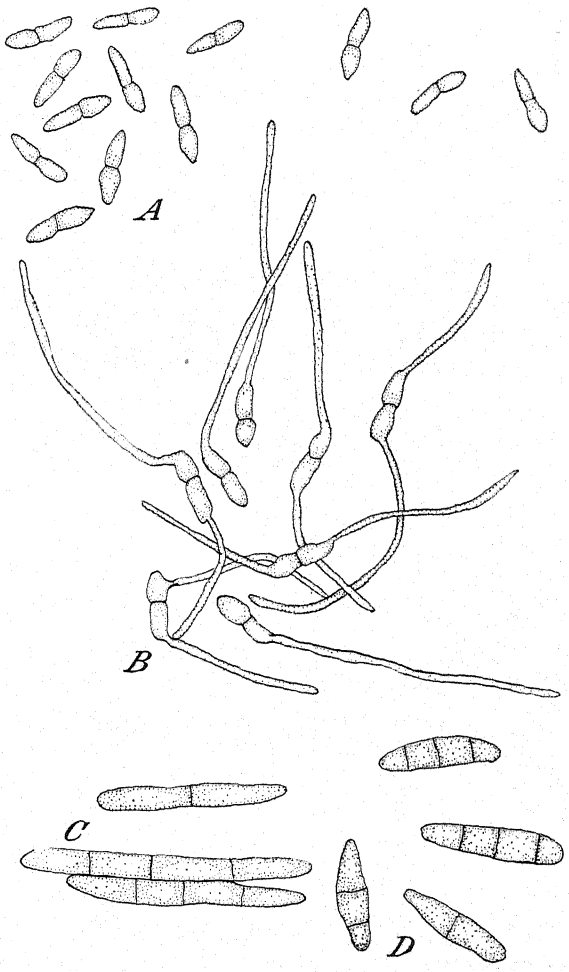


FIGURE 5.—*A*, Ascospores of *Mycosphaerella berkeleyii*; *B*, germinating ascospores after 15 hours in tap water; *C*, conidia from ascospore cultures; *D*, conidia from lesions obtained by inoculating peanut leaflets with conidia from ascospore cultures, 4 weeks after inoculation. All × 750.

GENETIC RELATION OF SPORE FORMS

Cultures and conidia produced on artificial media, from both ascospores and conidia, are identical in all measurable respects, except for the fact that the spores produced in culture are uniformly longer than those taken from peanut leaves and ascospores produce a slower initial growth than do conidia.

Tap-water suspensions of conidia, developed in culture from ascospores, were used as inoculum for inoculation tests, as well as conidia from conidial cultures and conidia from peanut leaves. Plants,

technique, and checks were as described earlier for *Mycosphaerella arachidicola*. The percentage of infection obtained was disappointingly low in those instances in which infection was obtained, but all lesions were identical with those on field material. The writer feels that these results may have been due to any one of several combinations of factors, including moisture, temperature, carbohydrate reserve of the plants used, as well as age of the plants. It is sufficient to say, however, that several inoculations produced typical infections, which yielded identical cultures when reisolated. The check remained free from infection. As a rule, infections were evident in from 10 days to 3 weeks, though some inoculations, in very hot weather, did not show symptoms of infection until after 4 weeks.

Further evidence of genetic relation was obtained from a study of sectioned material. In all cases thus far seen perithecia originate in the bases of conidiophore stromata, or are so close by that hyphal connections are perfectly evident. The lesion produced by the conidial stage is so characteristic and retains its identity so perfectly, that one can follow conidial, spermogonial, and perithecial development within the confines of lesions which are readily identifiable as being unmixed. This alone, in the absence of other data, would constitute weighty evidence of the genetic relation of these spore forms.

TAXONOMY

The form of the perithecia, the asci produced in fascicles, the absence of paraphyses, and the two-celled hyaline spores, are all clearly characteristic of the genus *Mycosphaerella* Johans. This species has apparently not been described previously. The writer proposes the designation *Mycosphaerella berkeleyii* in honor of the first author of this leaf spot fungus, with the following diagnosis:

Mycosphaerella berkeleyii sp. nov.

Syn.: *Cladosporium personatum* B. and C., 1875, Grev. 3: 106.
Cercospora personata (B. and C.) Ellis and Everhart, 1885, Jour. Mycol. 1: 63.
Septogloeum arachidis Rac., 1898, Ztschr. Pflanzenkrank. 8: 66–67.
Cercospora arachidis P. Henn., 1902, Hedwigia 41 (Beibl.): (15)–(18).

Perithecia scattered, mostly along margins of lesions produced by the conidial stage, amphigenous, partly embedded in host tissue, erumpent, broadly ovate to globose, 84μ–140μ×70μ–112μ, black, ostiolum slightly papillate; asci cylindrical club-shaped, short stipitate, fasciculate, 30μ–40μ×4μ–6μ, aparaphysate, bitunicate, eight-spored; spores uniseriate to imperfectly biseriate in the ascus, bicellular, the upper cell somewhat larger, slightly constricted at the septum, hyaline, 10.92μ–19.6μ×2.9μ–3.83μ (average 14.9μ×3.44μ).

Hab. In overwintered lesions produced by the conidial stage on leaflets of *Arachis hypogaea*, Experiment, Georgia, maturing during June and July.

Spermogonia: Scattered in and along borders of lesions produced by the conidial stage, ovate to globose, mostly heavy walls, black, amphigenous but perhaps more often epiphyllous, embedded in leaf tissue but later erumpent, ostiolate, 75μ–90μ×70μ–90μ; spermatia small, rod-shaped, hyaline, 1μ–3μ×0.5μ–1μ, arising endogenously, usually in fours within spermatiferous cells and liberated through sterigma-like processes.

On fallen leaflets, maturing throughout period from October through February.

Conidial stage: Spots mostly circular, often confluent, varying in size, 1 mm to several centimeters, brown to dark brown, often surrounded by a yellow halo on the upper surface when old, mostly on leaflets but often on stems late in season; conidiophores mostly hypophyllous, sometimes amphigenous when old, arising in more of less distinctly concentric tufts from heavy stromatic bases, fasciculate, geniculate, reddish-brown with mostly hyaline tips, continuous or one to several septate, 24μ–54μ×2μ–8.2μ; conidia somewhat obclavate but more generally cylindrical

with somewhat attenuated tips, pale brown to dilutely olivaceous, 18μ–60μ×5μ–11μ, one to eight septate, length and septations influenced by dry weather.

Parasitic on all above-ground parts of *Arachis hypogaea*, but mostly causing leaf spots.

Perithecíis sparsis, plerumque maculis marginatis, amphigenis, semiimmersis, punctiformibus, ovatis vel globosis, 84μ–140μ×70μ–112μ, nigris, ostiolis papillato praeditis; ascis cylindraceis clavatis, brevisme stipitatis, aparaphysatis, fasciculatis, bi-tunicatis, octosporis, 30μ–40μ×4μ–6μ; sporidiis uniseriatis vel biseriatis, bicellularibus, cellula superiore leniter latiore, leniter constrictis, hyalinis, 10.92μ–19.6μ×2.9μ–3.83μ plerumque 14.9μ×3.44μ.

Hab. in foliis dejectis *Arachidis hypogeae.*

Spermogoniis autumno efformatis, sparsis, plerumque maculis marginatis, ovatis vel globosis, nigris, amphigenis plerumque epiphyllis, innato-erumpentibus, punctiformibus, 75μ–90μ×70μ–90μ; spermatiis bacillaribus, hyalinis, 1μ–3μ×0.5μ–1μ.

Hab. in follis dejectis *Arachidis hypogeae.*

Statu conidico in maculis orbicularibus v. irregularibus, interdum confluentibus, magnis, brunneis vel atro-brunneis, plerumque ocraceis marginatis, plerumque foliis efformato; hyphis fertilibus hypophyllis, rare amphigenis, a stromate concentricis orientibus, fasciculatis, geniculatis, rubro-ferrugineis, sursum dilutioribus, continuis vel pluriseptatis, 24μ–54μ×2μ–8.2μ; conidiis sursum obclavatis plerumque cylindricis attenuatis apiciis, dilute brunneis vel dilute olivaceis, 18μ–60μ×5μ–11μ, one to eight septatis.

Hab. in foliis et ramis vivis *Arachidis hypogeae.*

For the convenience of plant pathologists and mycologists, type material of both species has been deposited in the following herbaria: Georgia Agricultural Experiment Station, Experiment, Ga.; Mycological Collections of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.; Plant Pathology Department, Cornell University, Ithaca, N. Y.

DISCUSSION

The disease caused by *Mycosphaerella arachidicola* usually appears at Experiment early in July; that caused by *M. berkeleyii* usually appears a few weeks later. By allowing for an incubation period of from 2 to 3 weeks, the time of appearance of the diseases in the field is closely correlated with the dates the organsims begin discharging ascospores. Primary infections are rarely so numerous as to attract much notice.

During the years these diseases have been under observation, *M. arachidicola* has been for the most part more widespread than *Mycosphaerella berkeleyii*. It also reaches epiphytotic proportions during August and early September, whereas *M. berkeleyii* is most destructive from September through harvest. During the epiphytotic months, the nights are cool and humid, a condition which especially favors the rapid spread of these organisms. Though both leaf spots occur on a given plant or a given leaflet, and even mixed lesions (i. e., lesions in which both organisms are fruiting) are not uncommon in the field, *M. arachidicola* usually causes the most damage to Spanish peanuts in Georgia owing to the fact that these varieties are usually harvested before *M. berkeleyii* reaches epiphytotic proportions. On other varieties on which comparisons can be made, *M. berkeleyii* usually causes a more rapid defoliation than *M. arachidicola.*

SUMMARY

The morphology and life history of the two peanut leaf spot fungi known heretofore as *Cercospora arachidicola* Hori and *C. personata* (B. and C.) E. and E., which cause destructive leaf spots of *Arachi-*

hypogaea (peanut), have been studied over a period of three seasons. Each fungus was found to produce spermogonia and perithecia, in addition to conidia.

Apparently neither perithecial stage has heretofore been described, and the author accordingly proposes *Mycosphaerella arachidicola* sp. nov. for the perithecial stage of *Cercospora arachidicola* and *M. berkeleyii* sp. nov. for the perithecial stage of *C. personata*.

The development of spermogonia and perithecia of both fungi is initiated during early fall. The spermogonia mature and (usually) cease liberating spermatia by February, whereas the perithecia do not mature until late in the following spring.

Additional evidence is presented to indicate that spermatia function as male sexual elements in the production of perithecia.

The disease caused by *Mycosphaerella arachidicola* reaches epiphytotic proportions during August and early September, whereas that produced by *M. berkeleyii* is most destructive from September through harvest.

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HOPI COTTON, A VARIABLE SPECIES [1]

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INTRODUCTION

There is much interest at present in Hopi cotton (*Gossypium hopi* Lewton), a species formerly cultivated by the Hopi Indians of northeastern Arizona on the Hopi Indian Reservation. This interest, stimulated by tests conducted by the Bureau of Agricultural Economics of the United States Department of Agriculture,[2] has resulted in requests for seed from cotton breeders in this and foreign countries, who hope to combine the spinning qualities of the aboriginal cotton with the productivity of commercial varieties. Archeologists are interested in Hopi cotton on account of its probable identity with the fiber found in prehistoric ruins in the Southwest.[3]

BREEDING STOCK

The behavior of several strains of *Gossypium hopi* in the breeding plots at the United States Field Station, Sacaton, Ariz., indicates that the species is genetically heterozygous and capable of modification by selection. Until 1933 only two strains were grown at Sacaton. One of these, "Moqui," is an inbred descendant of seed collected in 1911 at Oraibi Village, on the Hopi Indian Reservation, by Frank A. Thackery.[4] The other, "Sacaton Aboriginal", descended from seed obtained from a Pima Indian at Sacaton, on the Gila River Indian Reservation. It was assumed to be the kind of cotton grown by the Pimas before the advent of the white man. As grown at Sacaton, the plants of both strains are erect, 5 to 8 feet high, and have pitted bolls and yellow pollen. They differ in these characters from Lewton's description and illustrations of *G. hopi*.

In 1932 C. J. King and George J. Harrison, of the United States Department of Agriculture, obtained seed cotton from an old Hopi Indian at Moencopi, on the Hopi Indian Reservation, Ariz., who told them that he was growing his third successive crop for ceremonial purposes. Replies to previous inquiries about the production of cotton on the Indian reservations had indicated that none had been grown for a number of years.

In 1933 several hundred hills of cotton from this new supply of seed, designated "Hopi Moencopi" or "Hopi M," were grown at Sacaton. Approximately 40 plants were selected for propagation, several of which showed different degrees of pitting of the surface of

[1] Received for publication July 9, 1937; issued April 1938.

[2] ANONYMOUS. NEW FACTS ON STRENGTH OF COTTON START BREEDING FOR FINE SHORT STAPLE. Mid-South Cotton News 13 (11): 8. 1936.
CATES, J. S. NEW STAPLE BREEDING TARGET. Country Gent. 106 (9): 21, 84–85, illus. 1936.

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[4] LEWTON, F. L. THE COTTON OF THE HOPI INDIANS; A NEW SPECIES OF GOSSYPIUM. Smithsn. Misc. Collect. 60, no. 6, Pub. 2146, 10 pp., illus. 1912. (See p. 7.)

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the bolls. The progenies grown in 1934 from self-pollinated seed continued to show segregation in respect to boll surface, and further selections were made. In addition, plants with white corollas, and others with cream-colored pollen, were observed and selected. From these selections, progenies have been isolated that breed true for white corolla, yellow corolla, cream-colored pollen, yellow pollen, smooth boll surface (oil glands embedded and confined to an area near the sutures), and pitted boll surface. Figure 1 shows the range of pitting found in bolls of these progenies of the Hopi Moencopi strain.

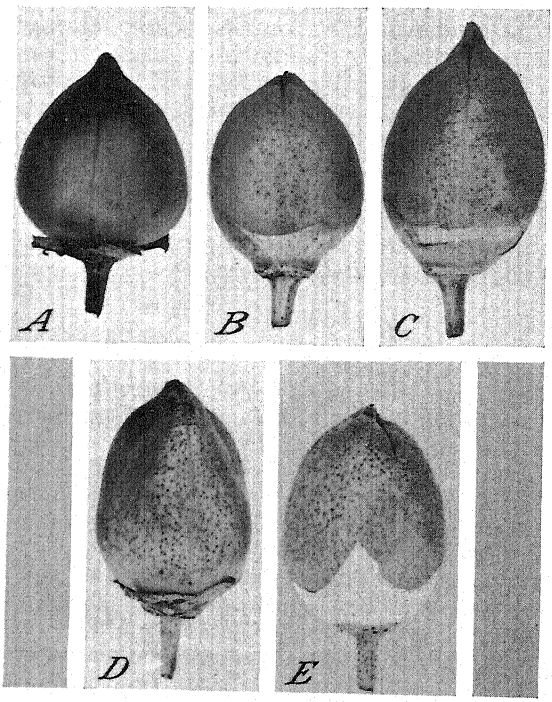


FIGURE 1.—Bolls from five progenies of the Moencopi strain of Hopi cotton showing differences in degree and character of the oil glands. The number of glands increases from none, or almost none, in *A* to numerous in *E*. In *A*, *B*, and *C* the glands, which are only along the sutures, are embedded, whereas in *D* and *E* they are in visible pits on the surface. Natural size.

In 1933 many of the plants exhibited the prostrate habit described by Lewton, having main stalks and limbs that reclined by the time they were 3 months old. In succeeding generations the progenies of plants from which self-pollinated seed was obtained tended to assume a more nearly erect habit. However, none of the plants in the progenies of the Moencopi strain have developed main stalks as strong as those of the Moqui and Sacaton aboriginal strains. This weakness of stems varied from progeny to progeny, being least pronounced in the progenies, grown in 1936, that were designated Hopi M 34–6–2 and M 34–9–2.

COMPARISON OF CHARACTERS

Table 1 presents a comparison of nine characters of the Hopi progenies grown at Sacaton in 1936. The first two progenies listed in the table, Hopi M 5–4–13 and M 5–4–10 (fig. 2), come nearest to matching Lewton's description, having yellow corollas, cream-colored pollen, and smooth boll surface. The next three selections from Hopi Moencopi have yellow instead of cream-colored pollen, and progenies M 34–6–2 and M 34–9–2 further depart from the original description by having the surface of the bolls conspicuously pitted like those of Pima (*Gossypium barbadense* L.). These two progenies approach the two long-inbred strains of Hopi—Moqui and Sacaton aboriginal—in plant habit, in corolla color, and in boll surface, and are the most productive

of the Moencopi strain. The last four progenies introduce another character, white corolla, and have boll surfaces intermediate in degree of pitting.

TABLE 1.—*Comparison of certain characters in progenies of Hopi cotton grown at the United States Field Station, Sacaton, Ariz., in 1936*

Progeny designation	Inbred generations	Corolla color	Pollen color	Boll surface grade [1]	Seed-cotton weight per boll	Seeds per boll	Seed index	Lint index	Lint percentage	Upper quartile fiber length
	No.				*Grams*	*No.*				*Inch*
Hopi M 5-4-13	3	Yellow	Light cream	0.15	1.63	16.1	8.05	2.00	20.0	0.93
Hopi M 5-4-10	3	do	Cream	.30	1.82	18.2	8.10	1.88	18.8	.96
Hopi M 6-10-1	3	do	Yellow	.94	1.26	12.6	7.70	2.26	22.8	.87
Hopi M 34-6-2	3	do	do	4.00	1.36	12.9	8.40	2.22	20.9	.98
Hopi M 34-9-2	3	do	do	4.00	1.40	13.8	7.90	2.18	21.9	.95
Moqui	13	do	do	3.75	1.95	16.5	10.04	1.78	15.2	.83
Sacaton aboriginal	14	do	do	3.75	2.04	20.0	8.62	1.54	15.6	.87
Hopi M 6-3-5	3	White	do	1.16	1.50	11.5	10.76	2.26	17.3	.88
Hopi M 6-14-1	3	do	do	1.96	1.79	15.6	9.00	2.48	21.6	.86
Hopi M 6-16-5	3	do	do	1.11	1.79	13.5	10.40	2.78	21.1	.84
Hopi M 6-16-7	3	do	do	1.34	1.65	13.2	9.98	2.56	20.5	.82

[1] Based on an arbitrary range of 5 grades, 0 to 4, inclusive, illustrated in fig. 1.

The undesirable character of Hopi cotton for commercial production is shown (table 1) by the seed-cotton weight per boll and the lint percentage. For the purpose of comparison, these and associated characters for Hopi and for Acala—the latter being the leading upland cotton variety grown in Arizona—are given in table 2.

TABLE 2.—*Comparison of certain characters in Hopi and Acala cottons*

Character	Hopi [1]	Acala
Seed-cotton weight per boll, grams	1.65	6.18
Seeds per boll, number	14.9	32.5
Seed index	9.0	13.0
Lint index	2.18	7.71
Lint percentage	19.6	36.0

[1] Means of the values for the respective characters listed in table 1.

In order to emphasize the small size of the Hopi bolls, it is computed that 1,403 would be required to furnish 1 pound of lint, as compared with only 204 bolls of the Acala variety. The values for seed index, lint index, and lint percentage show that the seeds of Hopi are not only small but are sparsely covered with lint.

The fiber length given in table 1 is the upper quartile of sorted arrays rather than the mean, as Webb [5] and his associates have found that in ginned cotton fiber the upper quartile length approximates the length determined by professional cotton classers. For fiber length Lewton [6] gives a range of 18 to 25 mm (0.70 to 0.98 inch). The Hopi progenies range from 0.82 to 0.98 of an inch in upper quartile length (table 1), and all of them seem to meet the requirements of the original description in having "white, strong, fine, and silky" lint.[6]

[5] WEBB, R. W. SUTER-WEBB COTTON FIBER DUPLEX SORTER . . . Amer. Soc. Testing Materials Proc. 35, v. 32, pt. 2, pp. 1–11, illus. 1932.

[6] LEWTON, F. L. See footnote 4, particularly p. 10 of reference.

Genetic experiments, involving interspecific crosses, confirm the evidence from morphological characters that *Gossypium hopi* is more closely related to *G. hirsutum* L. than to *G. barbadense*. Segregation in F_2 was much less pronounced in the cross with upland cotton (*G. hirsutum*) than in the cross with Egyptian cotton (*G. barbadense*),

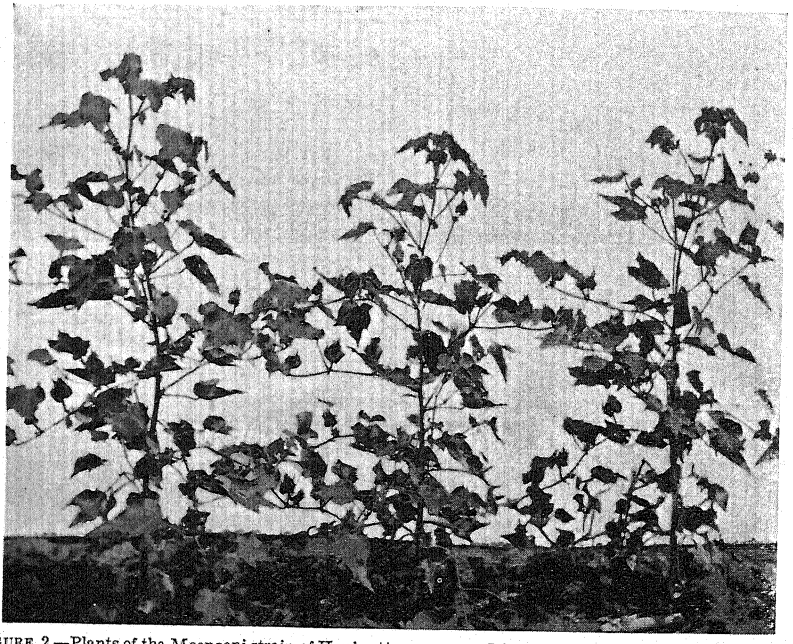


FIGURE 2.—Plants of the Moencopi strain of Hopi cotton, progeny M 5-4-10, 107 days after planting. Note the well-developed fruiting branches. The plants were from 30 to 34 inches high.

and there was much less sterility in *hirsutum* × *hopi* F_2 than in *barbadense* × *hopi* F_2. A study of the behavior of several contrasting characters in intraspecific crosses among different progenies of *G. hopi* is now in progress.

SUMMARY

The data presented in this paper indicate that Hopi cotton, formerly grown by the southwestern Indians, is heterozygous and capable of modification by selection. Progenies have been isolated that breed true for each character of the following allelomorphs: (1) Yellow and white corollas, (2) yellow and cream-colored pollen, and (3) smooth and pitted bolls.

OXIDASE AND CATALASE ACTIVITY OF BARTLETT PEARS IN RELATION TO MATURITY AND STORAGE[1]

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INTRODUCTION

In a previous publication (*3*) [2] it was pointed out that Bartlett pears picked relatively immature gave a less desirable canned product than did those left on the tree until more mature. The less mature fruit tended to have a pale orange-yellow color in contrast to the clear translucent cream color of the more mature fruit. This difference in color was due to changes in the fruit itself and not to differences in processing. It was found that for best results Bartlett pears should be harvested at a pressure test of 17 to 15 pounds (as measured on the pared flesh by means of the United States Department of Agriculture pressure tester with the $\frac{5}{16}$-inch plunger) and stored immediately at 30° to 32° F. for 15 to 30 days. Longer periods in cold storage tended to exaggerate the undesirable color of the less mature fruit.

An oxidase is believed to be the principal agent responsible for the darkening of pears in preparation for canning. In commercial canneries the pared fruit is placed in a sodium chloride solution to inactivate the enzyme and prevent excessive oxidation until the fruit is ready for processing. If the oxidase is very active the darkening will be rapid and the fruit may be discolored before it can be placed in the brine solution, or the enzyme may not be completely inactivated and the darkening may continue while the fruit is being graded and placed in the can.

The work reported herein was undertaken in the hope that information might be obtained on the oxidase and catalase activity of Bartlett pears that would be of value in reducing the wide variations in color in the canned fruit. The investigations were made at Wenatchee, Wash., during the 1935 and 1936 seasons.

REVIEW OF LITERATURE

In the study of the physiological development and storage of fruits, catalase has been used more often than oxidase to indicate metabolic changes; consequently the literature dealing with catalase is much more voluminous. However, oxidase activity probably plays a more important part in the discoloration of fruit during canning operations and is therefore of greater interest from the standpoint of food processing.

Ajon (*1*), working with citrus fruits, reported that as the fruit ripened the oxidase gradually diminished from the center of the parenchyma outward to the periphery, finally almost, if not quite,

[1] Received for publication August 18, 1937; issued April 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 345.

disappearing. De Villiers (*2*) also reported a decrease in oxidase activity of the grape as the berry ripened. Catalase increased with maturity but decreased slightly with final stages of maturation.

Haber (*7*) found greater oxidase activity in ripe tomato fruits than in green ones; catalase activity, on the other hand, was greater in green mature fruits. Gustafson et al. (*5*) reported a slightly lower catalase activity in yellow-orange and orange-colored tomatoes than in orange-red and red ones.

Lüers et al. (*11*) found that barley increased in catalase content during ripening until it became yellow, after which it decreased while the resting state was attained. During storage there was no change in the catalase content. Noguchi (*13*) reported a gradual decrease, without any sudden change at any time, in the catalase content of rice stored for 18 months. The oxidase content was unchanged.

Neller (*12*) found that in apples free from break-down catalase tended to increase during the earlier, then to decrease during later periods of storage, corresponding to the youth and senescence of the fruit. Harding (*8*) found that under cold-storage conditions an increase in catalase activity of Grimes Golden apples was a fairly accurate index to the approach of soggy break-down. Overholser (*14*) found that catalase decreased in Bartlett pears as maturity advanced and that "the effect of the storage temperature upon catalase activity depended upon the duration of the storage period, which varied with the temperature." Storage at 0° C. for 6 months resulted in greater activity in four varieties of pears. Reed (*15*) reported no catalase in green pineapples, some in hard yellow ones, and more in fully ripe ones.

In studies of the effect of maturity and storage on the oxidase and catalase activity of apples, Hinton (*10*) found that oxidase activity decreased during ripening on the tree, and markedly but slowly during storage. Catalase increased during storage, but in some cases decreased during the later stages. The rate of increase was highest during the early part of storage and fell more or less rapidly during the storage period. The later the date of picking the higher was the early rate of increase and the steeper the subsequent fall. With fruit picked at three stages of maturity the catalase activity was lowest in the middle picking. Hinton thought that this was probably due to lower temperatures prevailing prior to the second picking.

METHODS OF PROCEDURE

None of the fruit used in the present tests was canned. In order to make it possible to correlate oxidase and catalase activity with the previously observed behavior of the canned product, maturity and handling of the various lots of pears were similar to those reported in a previous paper (*3*). The oxidase activity was determined iodometrically as described by Guthrie (*6*) and is reported as cubic centimeters of N/100 sodium thiosulphate per 10 cc of juice, except as noted in the text. Catalase activity was determined by a water-displacement method similar to that described by Heinicke (*9*) and is reported as cubic centimeters of oxygen liberated in 5 minutes by 1 cc of juice. All enzyme determinations were made on the unripened fruit. Preliminary determinations made on the unripened fruit in storage at

32° F. and at daily intervals during ripening at 65° showed that the enzyme activity varied with the degree of ripeness. At the optimum stage for canning, oxidase activity was slightly greater than during storage at 32°, while catalase activity, which often increased at first, had fallen below that of the unripened fruit. In order to insure uniformity of sampling, unripened fruit was used. The methods of procedure have been described in detail by the authors in another paper (*4*).

EXPERIMENTAL DATA

EFFECT OF MATURITY ON ENZYME ACTIVITY

Bartlett pears were harvested at three stages of maturity, from three localities, and stored immediately at 32° F. Samples from each

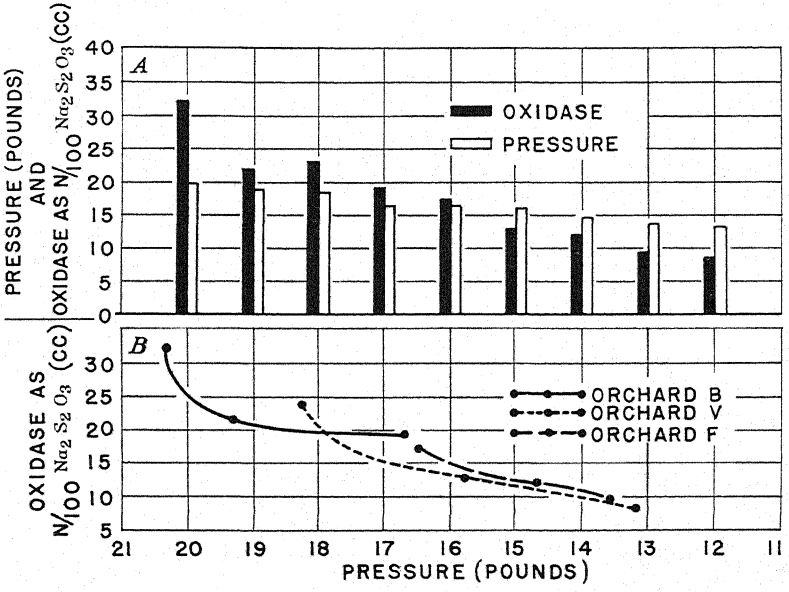


FIGURE 1.—Correlation between oxidase activity and maturity of nine lots of recently harvested Bartlett pears: *A*, Oxidase activity and pressure test of fruit from the three orchards arranged according to decreasing pressure; *B*, oxidase activity plotted against pressure test of fruit from individual orchards.

of the three localities were taken on the same dates, but owing to differences in elevation and soil conditions, the stages of maturity were not comparable. Oxidase and catalase determinations were made at harvest and at intervals during storage.

A very strong positive correlation was found between maturity, as measured by the pressure test, and oxidase activity, as is shown in figure 1. The pressure readings of the fruit during harvest ranged from 20.3 to 13.2 pounds, a drop of 35 percent, while the oxidase activity ranged from 32.8 to 7.9 cc of sodium thiosulphate, a drop of 76 percent.

No correlation was evident between degree of maturity, as measured by the pressure test, and catalase activity when the nine lots were arranged according to decreasing pressure. However, as shown in figure 2, when the lots were arranged by orchards the catalase activity

was low at the second picking and higher at the first and third pickings for two orchards. In the third orchard, in which the pressure reading was down to 16.5 pounds at the first picking, the catalase was low and continued to increase in the later pickings, which is in agreement with the hypothesis that catalase probably decreases as maturity advances, up to a definite stage, and then increases.

ORCHARD LOCATION AND ENZYME ACTIVITY

The orchards from which the pears used in the maturity studies were taken represent different growing conditions. Orchard F is a sandy loam, and fruit grown on this type of soil matures earlier than fruit grown on the type represented by orchard V, a medium-heavy loam more nearly typical of Wenatchee Valley orchards. Orchard B is also a medium-heavy loam but is situated at an elevation approximating 2,400 feet, whereas the elevation of the other two orchards is only 850 feet.

While the pears from the three orchards were not exactly comparable in maturity, there appear to be greater differences in enzyme activity than would be expected from maturity differences alone. For each pound drop in pressure there was an average drop in oxidase activity of 2.7 cc, 3.0 cc, and 3.8 cc in orchards F, V, and B, respectively, the greatest drop being in the least mature fruit. If the third picking of orchard B is compared with the first picking of orchard F and with the second picking of orchard V, at 16.7, 16.5, and 15.8 pounds, respectively—the three pickings most nearly comparable in maturity as measured by the pressure test—a difference will be noted of 0.85 cc of sodium thiosulphate in oxidase activity for each 0.1 pound difference in pressure between orchards B and F, 0.64 cc between orchards F and V, and 0.69 cc between orchards B and V. These values appear to be significantly higher than a corresponding difference in pressure would indicate in the individual orchards, where a maximum difference of 0.38 cc is noted for a similar drop in pressure. It thus appears that orchard differences would probably militate against setting an arbitrary oxidase figure for maturity (fig. 1, *B*). Seasonal conditions may also be a factor.

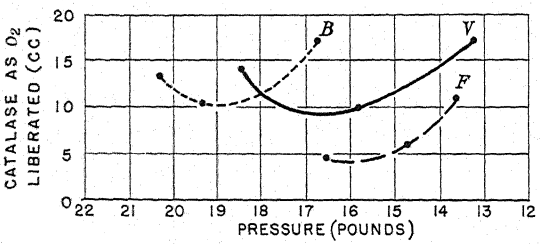


FIGURE 2.—Effect of maturity on catalase activity of Bartlett pears from different orchards.

EFFECT OF STORAGE ON ENZYME ACTIVITY

Bartlett pears from three orchards, harvested at three stages of maturity from each, were stored at 32° F. immediately after harvest. In table 1 are given the results of oxidase and catalase determinations at harvest and at intervals during storage. One lot, the first picking of orchard V, fluctuated irregularly throughout the storage period. This irregularity may have been due to spray materials on the fruit at harvest. With this exception, the oxidase and catalase activity were usually greater after storage than at harvest.

The effect of immaturity of fruit at harvest on its oxidase activity was magnified by holding the fruit in cold storage. This is shown graphically in figure 3, in which B (above) and V (below) represent fruit grown at elevations of about 2,400 and 850 feet, respectively. Fruit from orchard B was less mature than that picked on the same date from orchard V, and the oxidase increased more in storage. Also, the less mature fruit from orchard B showed a greater increase than the more mature fruit from the same orchard. It is significant that fruit harvested with a pressure test of 17 to 15 pounds (the pressure previously recommended for harvesting pears for canning), or below, showed comparatively little increase in oxidase activity during storage at 32° F. The greater oxidase activity in the early-picked fruit, especially after cold storage, accounts for the darker color observed in immature fruit when canned, and gives a basis for recommendations against holding immature Bartlett pears for long periods before canning. Catalase activity during storage was less closely associated with maturity at harvest than was oxidase activity.

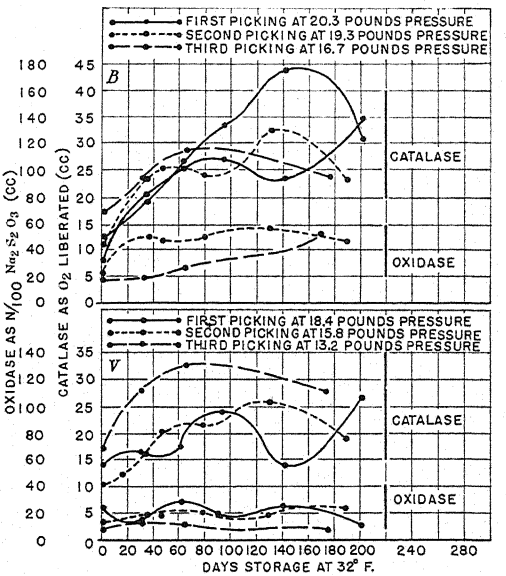


FIGURE 3.—Effect of maturity on oxidase and catalase activity of Bartlett pears at harvest and during storage. Orchard B at an elevation of about 2,400 feet; orchard V at an elevation of about 850 feet.

TABLE 1.—*Oxidase and catalase activity of Bartlett pears as influenced by maturity and storage*

Orchard	Pressure test [1]	Oxidase activity [2] after indicated number of days at 32° F.						Catalase activity [3] after indicated number of days at 32° F.					
		0	32	57	86	135	189	0	32	57	86	135	189
	Pounds	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
B	20.3	32.8	83.5	103.0	109.0	95.0	141.0	13.5	19.5	26.8	33.6	44.4	31.1
	19.3	21.8	50.5	47.0	50.0	57.0	46.5	10.6	22.0	25.6	24.4	32.6	23.9
	16.7	19.1	18.5	26.5	------	------	52.0	17.4	23.6	28.7	------	------	24.2
V	18.4	23.7	14.2	28.0	16.5	24.5	10.0	14.3	16.8	17.3	24.2	13.9	27.1
	15.8	12.9	19.5	18.0	20.0	18.5	23.5	10.3	16.3	20.2	21.6	26.0	19.4
	13.2	7.9	13.0	11.5	------	------	7.0	17.1	27.6	33.0	------	------	28.0
F	16.5	17.4	29.0	33.5	31.5	46.0	57.0	4.7	9.1	12.0	15.2	23.3	13.3
	14.7	11.8	17.5	15.0	18.0	25.0	20.0	6.3	10.0	11.0	14.5	16.8	9.1
	13.6	9.5	25.5	14.5	------	------	19.0	11.0	14.2	19.6	------	------	16.0

[1] Pounds pressure at harvest.
[2] N/100 sodium thiosulphate per 10 cc of juice.
[3] Oxygen liberated in 5 minutes by 1 cc of juice.

ENZYME ACTIVITY DURING THE GROWING SEASON

In 1936 the maturity studies were expanded to include a wider range of maturity and were limited to one orchard. Pear fruits were

harvested from the tree in orchard V that was used in 1935. Fruit for enzyme studies was picked June 11 and at intervals thereafter until September 9, when the pressure test was 11.6 pounds. During this time the average weight per fruit increased from 19.1 g to 270.5 g. The oxidase activity of the very immature fruit was so great that it became necessary to reduce the amount of juice to 1 cc for each determination. Otherwise the procedure was the same as in the preceding year. Samples for enzyme determinations were taken immediately after the fruit was harvested.

TABLE 2.—*Oxidase and catalase activity and rate of growth of Bartlett pears during the growing season in 1936*

Date sampled	Weight per fruit	Pressure test	Oxidase (N/100 sodium thiosulphate per 1 cc of juice)	Catalase (O_2 liberated in 5 minutes per 1 cc of juice)	Date sampled	Weight per fruit	Pressure test	Oxidase (N/100 sodium thiosulphate per 1 cc of juice)	Catalase (O_2 liberated in 5 minutes per 1 cc of juice)
	Grams	*Pounds*	*Cc*	*Cc*		*Grams*	*Pounds*	*Cc*	*Cc*
June 11	19.1	-------	24.0	24.0	Aug. 14	173.8	17.9	3.2	9.0
June 24	33.7	-------	13.6	17.4	Aug. 27	230.6	15.4	2.5	12.0
July 21	93.5	-------	6.2	9.8	Sept. 9	270.5	11.6	1.5	21.0
Aug. 4	151.0	-------	4.2	9.9					

The effect of maturity on enzyme activity is shown in table 2 and figure 4. Oxidase activity declined very rapidly during the early part of the season and decreased more slowly as the fruit approached maturity. Catalase activity decreased until a pressure test of 17.9 pounds was reached, and then increased until the activity at the close of the experiment approximated that of the first sampling.

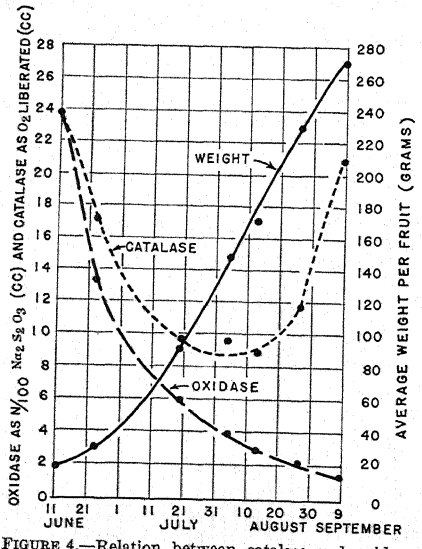


FIGURE 4.—Relation between catalase and oxidase activity and size of Bartlett pears during the growing season.

In the discussion of orchard location and enzyme activity it was pointed out that orchard differences would probably militate against setting an arbitrary oxidase figure as an index of maturity. In this connection it is of interest to compare the oxidase activity of the fruit from orchard V for the 2 years. Fruit taken from the same tree during 1935 and 1936, at pressure tests of 15.8 and 15.4 pounds, gave oxidase readings of 12.9 cc and 11.0 cc, respectively. The fruit was picked on August 26 in 1935 and on August 27 in 1936. The oxidase readings and the pressure tests indicate that the two seasons were fairly comparable. However, if the length of time from full bloom to harvest is taken as the index of maturity, the 1936 fruit, which was picked 122 days after full bloom, should have remained on the tree

6 days longer in order to reach the 128 days allowed the fruit in 1935. The oxidase activity would then have been less, and the difference in activity between the 2 years greater. While the difference in oxidase activity between the 2 years as given above may not be particularly significant, it appears unlikely that seasonal, orchard, and growing conditions will permit arbitrary oxidase limits to be set that will be applicable as a general index of the maturity at which Bartlett pears should be harvested.

The catalase activity on August 26, 1935, and August 27, 1936, was 10.3 cc and 12.0 cc, respectively. The former was the lowest reading recorded for the year 1935. Since samples were taken 12 days before and 17 days after the August 26 sampling, it may not be the absolute minimum for the year. In 1936 a minimum of 9.0 cc was recorded on August 14, and at time of sampling, on the 27th, the activity was increasing.

DISCUSSION

The decrease in oxidase activity in Bartlett pears as the fruit approached maturity is in agreement with the observed behavior of pears used for canning. Previously it was reported (*3*) that immature pears when canned gave a product grading toward a pale orange yellow in contrast to the clear cream yellow of more mature fruit. When the immature fruit was held in cold storage for long periods before ripening, the undesirable color increased. Practices that gave a dark, undesirable color in the canned product also gave increased oxidase activity, and practices that reduced darkening reduced oxidase activity. Where initial activity was low, it remained so during storage; but if the activity was high at the beginning of storage, it increased later so that differences in maturity were exaggerated by cold storage. The decrease in oxidase activity as the fruit approached maturity is in accord with the finding of Ajon (*1*) with citrus fruits, of De Villiers (*2*) with grapes, and of Hinton (*10*) with apples.

Catalase activity also was high in immature pears and decreased as the fruit approached harvest maturity. However, if the fruit was permitted to remain on the tree past the commercial harvest season catalase activity increased until the fruit was tree ripe, at which time the activity approximated that observed earlier in the season. Fruit picked at intervals representing early, medium, and late commercial maturity gave lower catalase activity at the second picking. These results were duplicated in different orchards and in different years, definitely indicating that the normal catalase curve of growing pear fruits is a U-shaped curve and that if the period of sampling were lengthened the extremities of the curve would presumably be extended.

The point of minimum activity of catalase varied in intensity and in relation to pressure test. This may be seen in figure 2, in which is shown the minimum for different orchards. Orchards B and V approached nearly the same minimum but reached it at different pressure tests. In orchard F the minimum was much lower. The minimum activity recorded for orchard V for the 2 years was 10.3 cc and 9.0 cc, respectively. If samples had been taken at shorter intervals a closer agreement between the minimum activity for the 2 years might possibly have been recorded.

While catalase activity does not appear to be correlated with color in the canned product it may be indirectly related to quality, since

the low point in the catalase curve occurs near the stage at which the fruit should be picked for canning in order to secure best results.

The low point in the catalase curve may also indicate a critical stage in the metabolism of the fruit, since it coincides with the period of minimum respiratory intensity (*4*) and also with the pressure test usually recommended in the Wenatchee district for the harvesting of Bartlett pears for best dessert and storage quality.

These results with pears lead the writers to suspect that the catalase curve of apples may also be a U-shaped curve and that the low results reported by Hinton (*10*) in his middle picking of apples may have been due to this characteristic of the catalase activity rather than to the cold weather to which he ascribed his results.

That the low points in the catalase curves in the present work are not due to low temperatures just before harvest may be seen from an examination of figure 2, which shows that fruit from different orchards, exposed to similar temperatures, reached the minimum in catalase activity at different times. Furthermore, in figure 4 the mean temperatures for the 7 weeks preceding harvest were 62°, 69°, 75°, 76°, 76°, 66°, and 70° F., respectively. Any correlation that might be found in this case between temperature and catalase activity of the fruit while still on the tree would evidently be a negative one.

It is not particularly surprising that the catalase curve should be U-shaped. Overholser (*14*) reported that immature Bartlett pears were high in catalase, which decreased as maturity advanced. His results indicated a continuous decrease. However, a study of his data permits some interesting comparisons to be made. Since it is not known how mature his fruits were at the time of final sampling, it may be possible that he discontinued sampling before the rise in catalase began. This hypothesis is supported by the fact that on May 28 the average weight of his pears was 15.2 g; on June 11 the average weight of the authors' pears was 19.1 g. Assuming that the rate of growth before the first sampling was approximately equal in the two cases, then the season of his study was about 2 weeks earlier than the season in which this study was made. If this is true, he discontinued sampling approximately 110 days after full bloom. The minimum activity recorded in the present study was 109 days after full bloom. If the number of days from full bloom be taken as the measure of maturity, he discontinued sampling at the stage of maturity at which the least activity was found in the work covered by this paper, and his results might be said to agree with those presented here. His report of 8.6 cc and 8.4 cc of oxygen liberated on July 27 and August 4, respectively, would indicate the low points in the catalase curve had been reached. Overholser mentioned that his results did not agree with Reed's findings of increase of catalase activity with ripening in the case of pineapples, when green, hard yellow, and fully ripe fruits were used.

When Bartlett pears are permitted to remain on the tree until tree-ripe, there is a tendency for the flesh to break down at the core. Harding (*8*) and Neller (*12*) have reported increased catalase activity of apples in storage as they approach the break-down stage. It is possible that the accumulation of acetaldehyde and alcohol which takes place as fruit approaches the break-down stage stimulates catalase production, causing the rise in activity. This viewpoint is strengthened by the increase in catalase activity of fruit subjected to alcohol vapors (*4*).

SUMMARY

The effect of maturity and handling practices on the oxidase and catalase activity of Bartlett pears has been studied in relation to the observed behavior of pears used for canning.

Oxidase activity decreased throughout the growing season.

The catalase activity of Bartlett pears from the time they were very small until they were tree-ripe formed a U-shaped curve. The minimum activity occurred near the period at which the fruit should be harvested for canning.

High oxidase activity is correlated with practices that give an undesirable color in canned pears, and low oxidase activity is correlated with practices that give a desirable color. It therefore appears that high oxidase activity may be the principal cause of poor color in the canned product.

Catalase activity apparently is unrelated to color development in canned Bartlett pears but may be related indirectly to quality since best results are obtained with fruit picked when the low point in the catalase curve is reached or soon thereafter.

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A COMPARATIVE STUDY OF AN EARLY, A MEDIUM, AND A LATE STRAIN OF TIMOTHY HARVESTED AT VARIOUS STAGES OF DEVELOPMENT [1]

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INTRODUCTION

In Ohio, timothy and timothy mixtures make up the largest percentages of the hay crop. In 1934, 25.5 percent of the hay acreage harvested was timothy, and 37.4 percent was timothy-clover mixture.[2]

At the Missouri Agricultural Experiment Station it was found that the palatability of timothy fed to steers was greater when the crop was cut early than when it was cut nearly mature (*10*),[3] the order of preference shown by the steers for the hay being the same as the order in which the timothy was cut.

At the Ohio Agricultural Experiment Station (*7*) it was found that the amounts of both vitamin B and vitamin G are positively correlated with leafiness, greenness, and the protein content of alfalfa, clover, and timothy plants, and that these vitamins decrease as the season advances.

In an experiment conducted with sheep at the Ohio station (*2, p. 179*) it was found that—

> the time at which timothy was cut for hay had a definite influence on its value for sheep feeding. Timothy cut when not more than one-third of the heads were in blossom was fully one-third better than timothy cut after the blossoms had fallen and as the seed was ripening. From a practical standpoint, the early cut timothy was a usable roughage whereas the late cut * * * was decidedly inferior.

With few exceptions, time-of-harvesting and feeding experiments have been carried on with ordinary unimproved timothy (*Phleum pratense* L.). The development of improved varieties or strains at the Timothy Breeding Station at North Ridgeville, Ohio (*3*),[4] and at other agricultural experiment stations in the United States and elsewhere, and their probable distribution to farmers, made it desirable to compare certain ones, especially early and late strains, with ordinary timothy in a time-of-harvesting experiment. The results of such an experiment are reported in this paper.

MATERIALS AND METHODS

STRAINS USED

The timothy strains used in this experiment were F. C. 11901,[5] a medium early maturing selection; F. C. 12368, a late-maturing selec-

[1] Received for publication June 1, 1937; issued April 1938. Contribution from the Division of Forage Crops and Diseases, U. S. Department of Agriculture, cooperating with the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] The authors are indebted to Dr. J. I. Falconer, chairman of the Department of Rural Economics, Ohio State University, for these data.

[3] Reference is made by number (italic) to Literature Cited, p. 363.

[4] The Division of Forage Crops and Diseases; Bureau of Plant Industry, U. S. Department of Agriculture, and the Ohio Agricultural Experiment Station cooperating.

[5] Accession number of the Division of Forage Crops and Diseases. If introduced into production, F. C. 11901 will be known as Marietta timothy.

tion; and the ordinary unimproved timothy grown in this country and designated herein as medium timothy. The selections were developed by the senior author at the Timothy Breeding Station where the time-of-harvesting experiment was made.

At North Ridgeville in northern Ohio, medium timothy is usually in full bloom from about June 22 to July 1. The early strain is about 4 days earlier and the late strain about 7 days later than medium timothy.

PLOT TECHNIQUE

The land was plowed in the summer of 1930, and late in August an application of 180 pounds per acre each of nitrate of soda and 16-percent superphosphate was applied to the seedbed. On September 18, plots of the three strains of timothy were sown at the rate of 5 pounds of seed per acre with a nurse crop of rye. Medium red clover, at the rate of 10 pounds per acre, was sown on all plots the following spring. The rye was harvested for grain in 1931. The individual plots from which hay was harvested were 49.5 by 5 feet. There were 24 plots of each of the three strains of timothy, quadruplicate plots being harvested at each of six harvest periods.

The six harvests were made during a period of 35 to 38 days, the first one being made when medium timothy was partly headed and the last one when its seed was mature. The six periods have been designated by the letters A to F, respectively. The actual dates of harvest, together with the condition of the timothy, are shown in table 1. All harvests were made according to this schedule except the aftermath of 1932, which was harvested on August 31.

TABLE 1.—*Date of harvesting and condition of each strain, 1932–34*

[Time of harvest was determined by the condition of ordinary timothy]

Year	Period of harvest	Date of harvest	Condition of indicated strain of timothy		
			F. C. 11901 (early)	Ordinary (medium)	F. C. 12368 (late)
1932 [1]	A	June 14	Headed	Partly headed	No heads.
	B	June 18	Full bloom	Fully headed	Few heads.
	C	June 22	do	Early bloom	Nearly headed.
	D	July 1	Past bloom	Just past full bloom	Full bloom.
	E	July 12	60 percent heads becoming straw color.	Seed beginning to mature.[2]	Just past full bloom.
	F [3]	July 20	Mature	Seed mature [4]	50 percent heads becoming straw color.
1933	A	June 9	Nearly headed	Partly headed	No heads.
	B	June 14	Early bloom	Fully headed	Few heads.
	C	June 20	Full bloom	Early bloom	20 percent heads emerged.
	D	June 29	Past bloom	Just past full bloom	Early bloom.
	E	July 8	85 percent heads becoming straw color.	Seed beginning to mature.[2]	Past bloom.
	F	July 17	Mature	Seed mature [4]	30 percent heads straw color.
1934	A	June 11	Very early bloom	Partly headed	No heads.
	B	June 14	Early bloom	Fully headed	Few heads.
	C	June 19	Full bloom	Early bloom	Nearly headed.
	D	June 28	Past bloom	Just past full bloom	Early bloom.
	E	July 9	90 percent heads becoming straw color.	Seed beginning to mature.[2]	Past full bloom.
	F	July 16	Mature	Seed mature [4]	25 percent heads straw color.

[1] The crop of aftermath obtained in 1932 was harvested on Aug. 31.
[2] About 10 percent of the earliest heads beginning to turn straw color.
[3] At the last date of harvesting the main crop of mixed hay in 1932, most of the clover heads and a large proportion of the lower leaves on the clover stems were dry and brown.
[4] Nearly all heads straw color.

The percentages of timothy, clover, and weeds in the dry hay were determined for the 1932 and 1933 crops by sorting representative square-yard samples from each plot; the 1934 crop of clear timothy required no sorting.

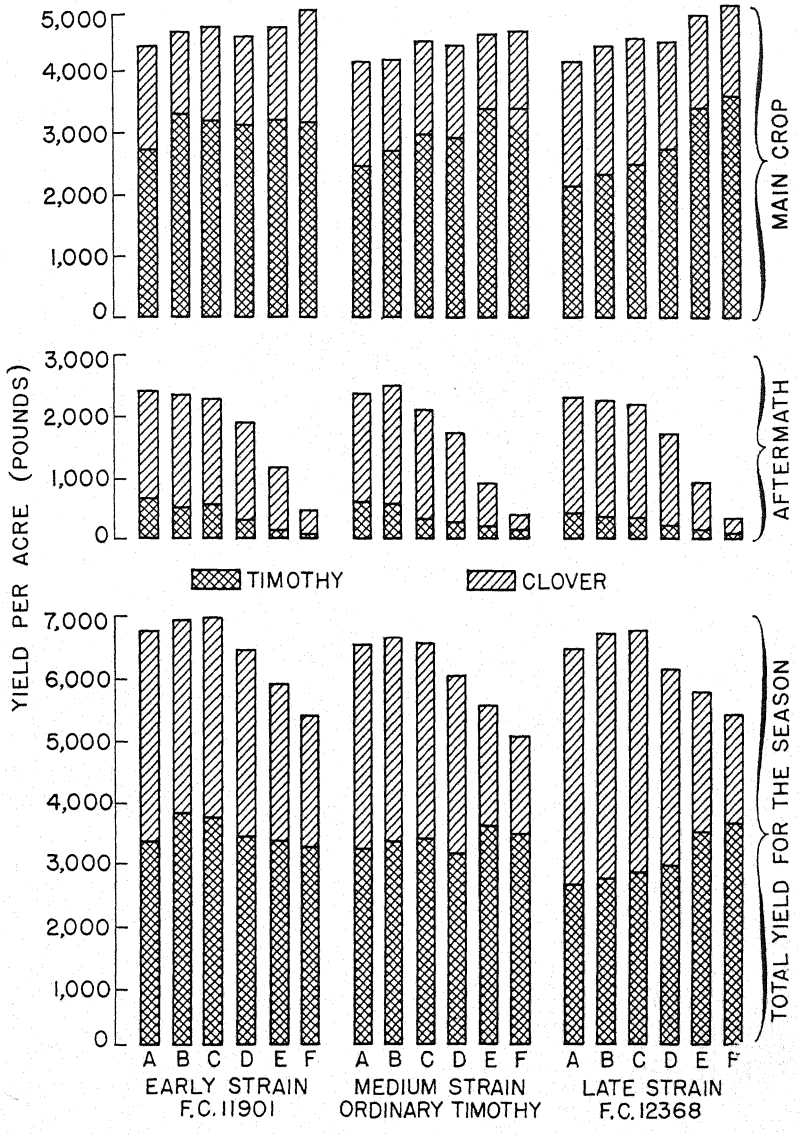


FIGURE 1.—Yields of timothy and of clover, in the main crop and the aftermath, and the total in both crops harvested in 1932. Letters at bottom indicate harvesting periods shown in table 1.

The protein (nitrogen×6.25) content of the hay as harvested and as sorted was determined by the Department of Agronomy of the Ohio Agricultural Experiment Station.

On May 10, 1934 (the third harvest season), 150 pounds per acre each of sulphate of ammonia and 20-percent superphosphate were applied as a top dressing on the timothy.

INTERPRETING THE DATA

The data obtained consisted of the yields of hay, the proportions of timothy and clover in the mixed hay, the percentages and yields of protein, and the color analyses and grades of hay. In interpreting these data, the following facts should be borne in mind:

(1) Because of the differences in their response to days of various lengths (*4*) early, medium, and late timothy growing in different

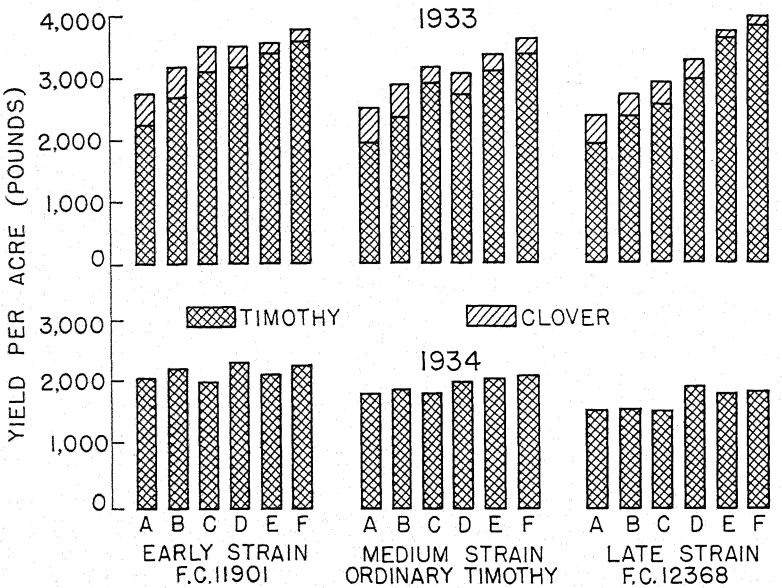


FIGURE 2.—Yields of timothy and clover hay in 1933, and of timothy in 1934.

latitudes (*5*) vary in relative dates of heading and of blooming and period of development, and caution should be used in predicting the probable results to be expected at locations north or south of North Ridgeville.[6] Experiments so far conducted indicate that in general early varieties of timothy are well adapted to both southern and northern latitudes within the range where timothy is grown, whereas late varieties are adapted to northern latitudes only.

(2) The three strains of timothy were grown in mixture with medium red clover (*Trifolium pratense* L.). There is no assurance that yields comparable to those obtained could have been expected had the clover been omitted. Nilsson-Leissner (*9*), in Sweden, found that the growth response of any variety of timothy grown in mixture with clover may be entirely different from that of the same variety grown alone. However, the yields of the timothy fractions of the hay may give some indication, at least, of what may be expected in the absence of a companion crop of clover.

[6] North Ridgeville is located at lat. 41°23′ N.

(3) The amount and distribution of the rainfall during the spring and early summer have an influence upon the yields of timothy. The yield in 1934 was undoubtedly reduced by the abnormally dry weather during May and the first half of June. Table 2 shows the records of precipitation at the Cleveland, Ohio, station of the Weather Bureau, United States Department of Agriculture, which is located about 20 miles east of the Timothy Breeding Station at North Ridgeville and at approximately the same elevation.

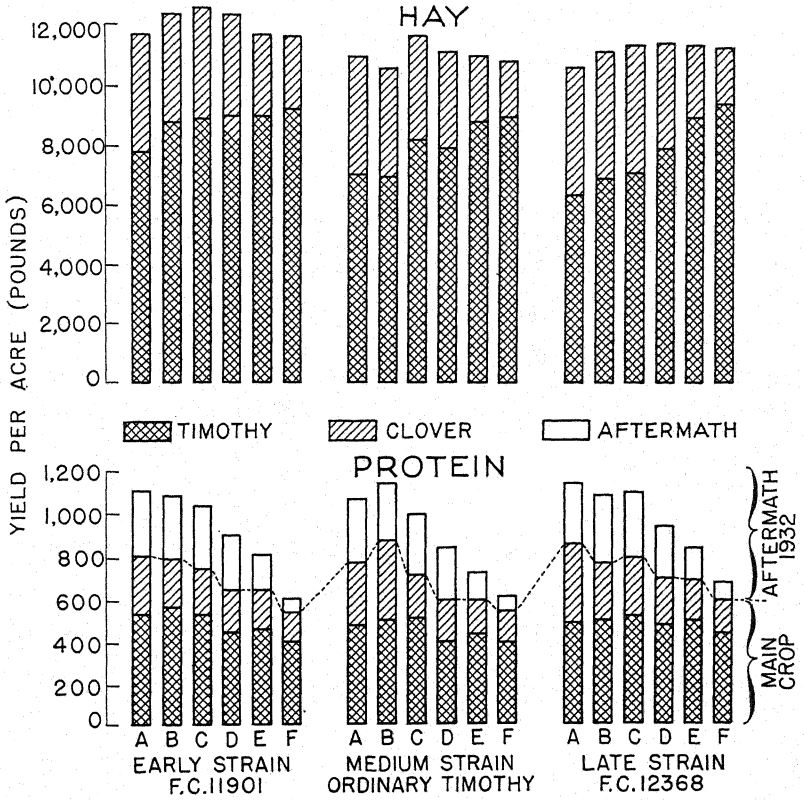


FIGURE 3.—Total yields of hay and yields of protein produced by the timothy and by the clover in 3 years.

TABLE 2.—*Monthly and annual precipitation at the United States Weather Bureau station at Cleveland, Ohio*

Year	Monthly precipitation												Annual precipitation
	January	February	March	April	May	June	July	August	September	October	November	December	
	In.	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*
1932	3.46	1.10	3.39	2.09	3.71	1.88	2.42	4.31	1.45	3.06	2.63	3.77	33.27
1933	1.40	1.99	3.51	2.42	2.86	.39	1.26	1.98	2.73	.97	3.06	1.60	24.17
1934	1.58	1.02	2.43	2.43	.58	[1]2.26	2.70	2.46	2.12	.99	1.40	1.84	21.81
Mean, 1871 to 1935	2.54	2.38	2.75	2.47	3.00	3.16	3.45	2.90	3.21	2.70	2.57	2.40	33.53

[1] In June 1934, 97.4 percent of the rainfall occurred on and after June 18.

TABLE 3.—*Yields per acre of hay, 1932–34*

Year	Crop	Yield per acre at indicated harvest period (main crop)																	
		Early timothy (F. C. 11901)						Medium timothy (ordinary variety)						Late timothy (F. C. 12368)					
		A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
		Lb.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
1932	Main crop:																		
	Timothy fraction [1]	2,735	3,317	3,188	3,143	3,227	3,196	2,565	2,812	3,025	2,852	3,419	3,407	2,155	2,367	2,608	2,778	3,365	3,582
	Clover fraction	1,703	1,336	1,543	1,472	1,528	1,830	1,601	1,431	1,490	1,492	1,235	1,315	2,034	2,099	2,091	1,779	1,552	1,529
	Total (mixed hay)	4,438	4,653	4,731	4,615	4,755	5,026	4,166	4,243	4,515	4,344	4,654	4,722	4,189	4,466	4,699	4,557	4,917	5,111
	Aftermath: [2]																		
	Timothy fraction [1]	645	465	542	281	138	62	629	545	383	299	203	76	435	361	335	190	150	79
	Clover fraction	1,771	1,855	1,757	1,611	1,049	360	1,766	1,950	1,747	1,438	738	294	1,885	1,953	1,875	1,519	768	278
	Total (mixed hay)	2,416	2,320	2,299	1,892	1,187	422	2,395	2,495	2,130	1,737	941	370	2,320	2,314	2,210	1,709	918	357
1933	Main crop (only):																		
	Timothy fraction [1]	2,225	2,692	3,086	3,154	3,374	3,584	1,916	2,343	2,856	2,671	3,065	3,350	1,886	2,351	2,541	2,932	3,599	3,791
	Clover fraction	488	462	378	303	187	193	568	522	266	368	239	223	463	332	321	277	141	162
	Total (mixed hay)	2,713	3,154	3,464	3,457	3,561	3,777	2,484	2,865	3,122	3,039	3,304	3,573	2,349	2,683	2,862	3,209	3,740	3,953
1934	Main crop (only): Timothy	2,095	2,248	2,046	2,343	2,152	2,284	1,830	1,922	1,841	2,001	2,049	2,070	1,674	1,668	1,659	1,915	1,798	1,857
1933–34	Total	11,662	12,375	12,540	12,307	11,655	11,509	10,875	11,525	11,608	11,121	10,948	10,735	10,532	11,131	11,430	11,390	11,373	11,278
	Total minus aftermath [3] in 1932	9,246	10,055	10,241	10,415	10,468	11,087	8,480	9,030	9,478	9,384	10,007	10,365	8,212	8,817	9,220	9,681	10,455	10,921

[1] Includes the small percentage of weeds.
[2] Harvested all on one date, Aug. 31.
[3] It is assumed that if the aftermath in 1932 had not been harvested there would have been no change in the yields in 1933 and 1934.

YIELDS OF HAY

The yields of hay are recorded in table 3 and illustrated graphically in figures 1, 2, and 3. The first season's crop of 1932 consisted of a main crop, of mixed timothy and clover, and an aftermath, mostly clover. The following year, 1933, the main crop of mixed hay contained only a small amount of clover, and the third year's crop was clear timothy.

In the main crop of mixed hay, except the plots of early timothy in 1932, the yields of the clover fraction were less for the last two harvest periods than for the earlier ones. This loss may have been due to the

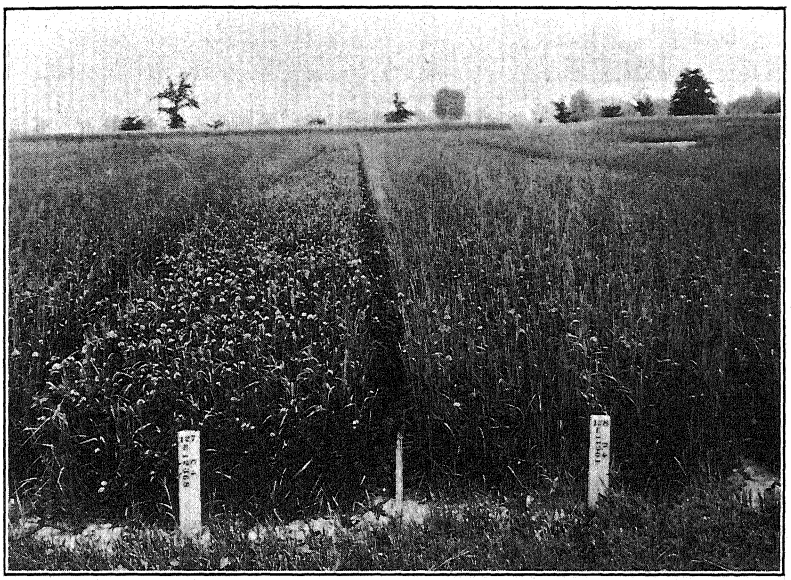


FIGURE 4.—Plots of timothy and clover mixture. Right, early timothy (F. C. 11901) in early bloom. Left late timothy (F. C. 12368) only partly headed. Photographed June 16, 1932.

shattering of the leaves and other parts of the clover plants as they approached maturity (*11*).

The time of harvesting the main crop had a very marked effect upon the yields of the aftermath harvested August 31, the yields falling off most rapidly when the main crop was harvested after the C period (June 22). If the aftermath is included in the total yields of hay for the season, the advantage of early harvesting is evident, as shown in figure 1.

In 1933 (fig. 2), as in the main crop of 1932, the yields increased as harvesting took place later. Since in 1933 the crop consisted largely of timothy, late harvesting gave larger increases in yield than in 1932.

In 1934 (fig. 2) the effect of time of harvest on yield was less marked than in 1932 and 1933. The dry weather during May and June limited the growth in all plots. The early timothy suffered less from the drought than did the late timothy. Apparently the relatively early growth of the culms and the earlier heading and blooming of the early timothy enabled it to more nearly complete a normal growth before soil moisture became too much of a limiting factor. Had the

rainfall been normal it is probable the late timothy would have made higher yields at the D, E, and F periods of harvest. Later harvesting resulted in higher yields in all three timothy strains when the aftermath in 1932 was deducted from the total yields for 3 years (table 3).

The total yields of hay for the three seasons—aftermath included—are shown in figure 3. The early timothy produced more hay than either the medium or the late timothy, especially at the early periods of harvest. The total yields produced by the early strain for all 3

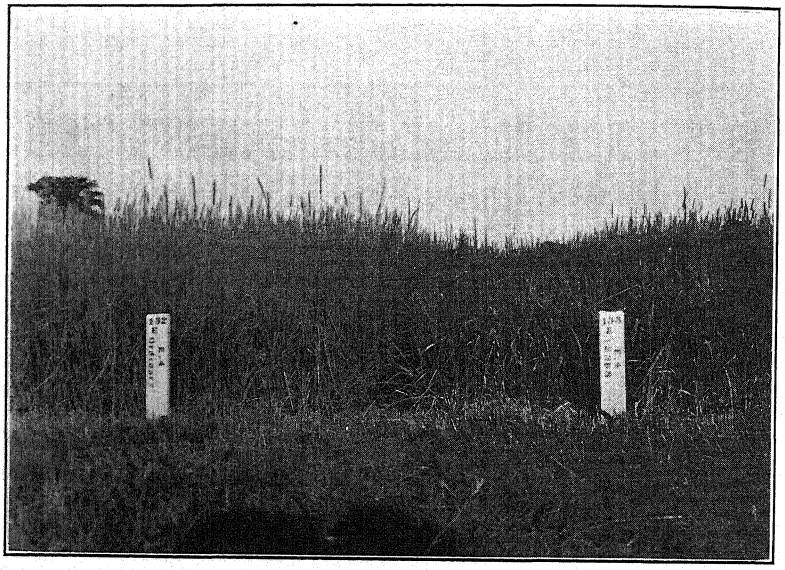


FIGURE 5.—Right, late timothy (F. C. 12368), maximum height 27 inches. Left, medium timothy (ordinary), maximum height 36 inches. Photographed June 21, 1933.

years, at comparable periods of harvest, were about 700 to nearly 1,200 pounds per acre greater than those produced by ordinary timothy.

Table 3 shows that in the first four periods of harvest of the main crop in 1932 there was an average of 487 pounds more clover per acre with the late than with the early timothy, and 497 pounds more with the late than with the ordinary timothy. This may be attributed to the relatively slow growth of the late timothy. Since clover adds to the feeding value of the mixed hay this may be a point in favor of late timothy (figs. 4 and 5).

PROPORTIONS OF DIFFERENT CONSTITUENTS IN THE HAY

Table 4 shows that in the first or main crop harvested in 1932, at all but the last two harvest periods, there was a larger percentage of clover in the late selection than in either the early selection or the medium timothy, which were not greatly different. Delaying the harvest of the ordinary and late timothy tended to decrease the percentage of clover in the first crop, but this is not apparent for the early timothy, possibly because of some error.

TABLE 4.—*Proportions of timothy, clover, and weeds in the two crops harvested in 1932 and in the crop harvested in 1933*

Year	Crop	Constituent at indicated harvest period [1] (main crop) of—																	
		Early timothy (F. C. 11901)						Medium timothy (ordinary variety)						Late timothy (F. C. 12368)					
		A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1932	Main crop:																		
	Timothy	61.6	70.5	67.1	68.0	67.5	63.5	61.5	66.2	66.8	65.6	73.4	71.9	51.3	52.8	54.5	60.4	68.0	69.5
	Clover	38.4	28.7	32.6	31.9	32.1	36.4	38.4	33.7	33.0	34.3	26.5	27.8	48.6	47.0	44.5	39.0	31.6	29.9
	Weeds	.0	.8	.3	.1	.4	.1	.1	.1	.2	.1	.1	.3	.1	.2	1.0	.6	.4	.6
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Aftermath: [2]																		
	Timothy	26.3	19.4	23.6	14.4	11.3	14.7	26.1	21.7	17.5	17.1	21.3	20.5	18.6	15.3	14.4	11.0	15.7	22.1
	Clover	73.3	80.0	76.4	85.2	88.4	85.3	73.7	78.2	82.0	82.8	78.4	79.5	81.3	84.4	84.8	88.8	83.6	77.9
	Weeds	.4	.6	.0	.4	.3	.0	.2	.1	.5	.1	.3	.0	.1	.3	.8	.2	.7	.0
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1933	Main crop (only):																		
	Timothy	81.0	84.7	88.8	90.9	94.4	94.8	76.2	81.5	91.3	87.3	92.6	93.2	79.6	86.8	87.7	90.3	95.3	95.0
	Clover	18.0	14.7	10.9	8.8	5.3	5.1	22.9	18.2	8.5	12.1	7.2	6.2	19.7	12.4	11.2	8.6	3.8	4.1
	Weeds	1.0	.6	.3	.3	.3	.1	.9	.3	.2	.6	.2	.6	.7	.8	1.1	1.1	.9	.9
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] See table 1 for explanation of harvest periods.
[2] Harvested all on 1 date, Aug. 31.

In the second crop, or aftermath, harvested in 1932, and also in the crop of 1933, the differences in the percentage of timothy and clover for the different varieties show no consistent trends.

PROTEIN CONTENT OF THE HAY

The percentage of protein in the hay, and consequently its feeding value, was markedly influenced by the time of harvest.

The percentage of protein in the mixed hay, and also in the timothy and clover fractions, in the main crop of 1932 and in the crop of 1933, together with the percentages of protein in the timothy harvested in 1934, are presented in table 5 and are shown graphically in figures 6 to 10. The calculated yields of protein per acre are also presented in table 5 and are shown graphically in figures 3 and 11.

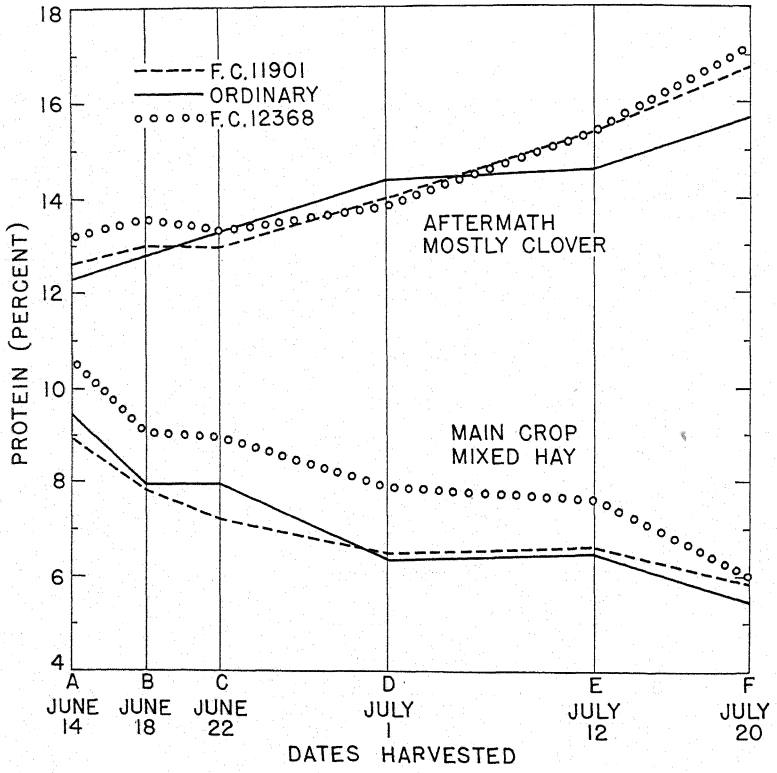


FIGURE 6.—Percentages of protein in the mixed hay produced by the main crop and by the aftermath in 1932.

PERCENTAGE OF PROTEIN

In both the timothy and the clover, the percentages of protein in all of the main crops were highest in the hay harvested at the earliest dates and gradually decreased, with minor exceptions in occasional samples, up to the time of latest harvest.

In each season, the percentage of protein in the mixed hay, in the timothy fraction of the mixed hay, and in the timothy alone, at any harvest period, differed but little for the early and medium timothy. This may be attributed to the tendency of the leaves on the stems of F. C. 11901 to remain almost as green as those of ordinary timothy (*6*) at comparable periods of harvest.

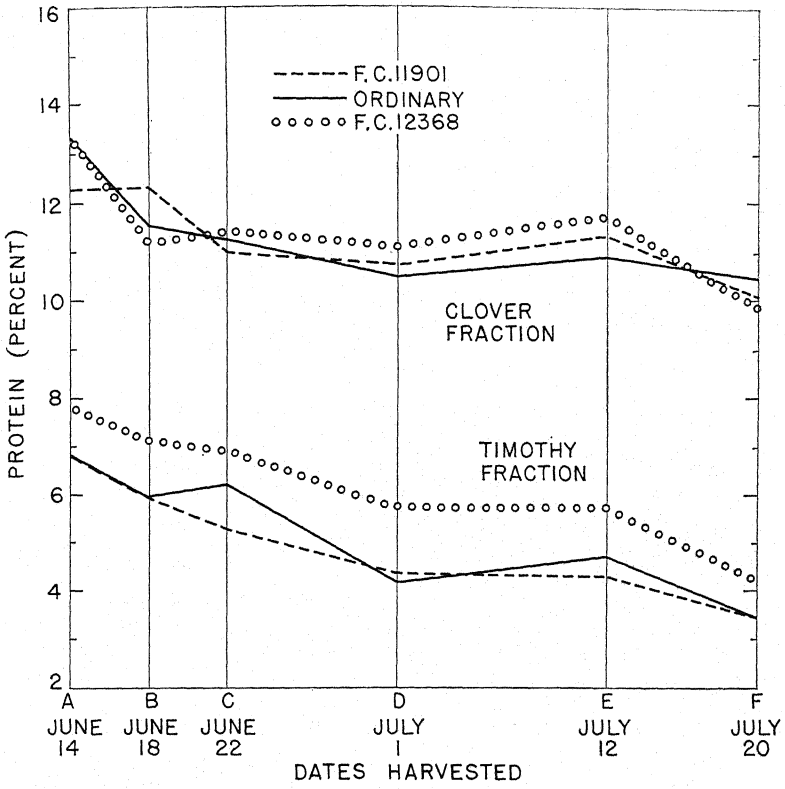


FIGURE 7.—Percentages of protein in the timothy and clover fractions of the main crop in 1932.

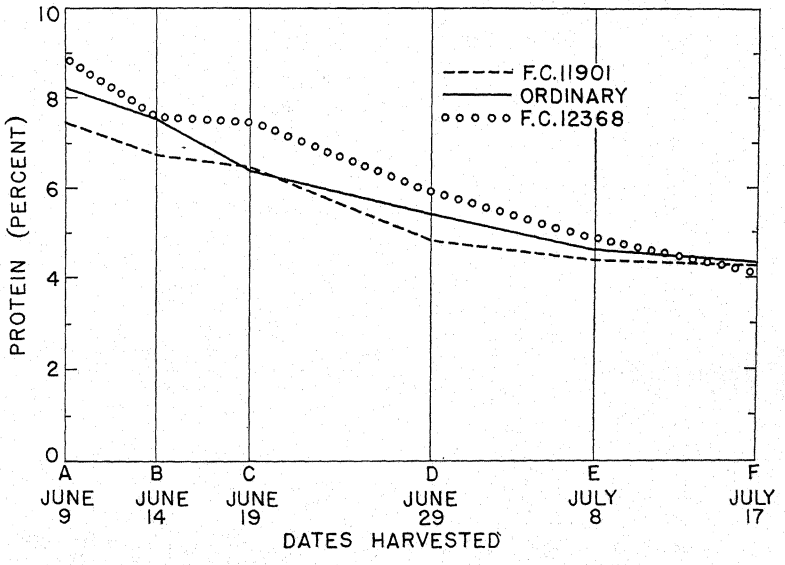


FIGURE 8.—Percentages of protein in the 1933 crop of mixed hay.

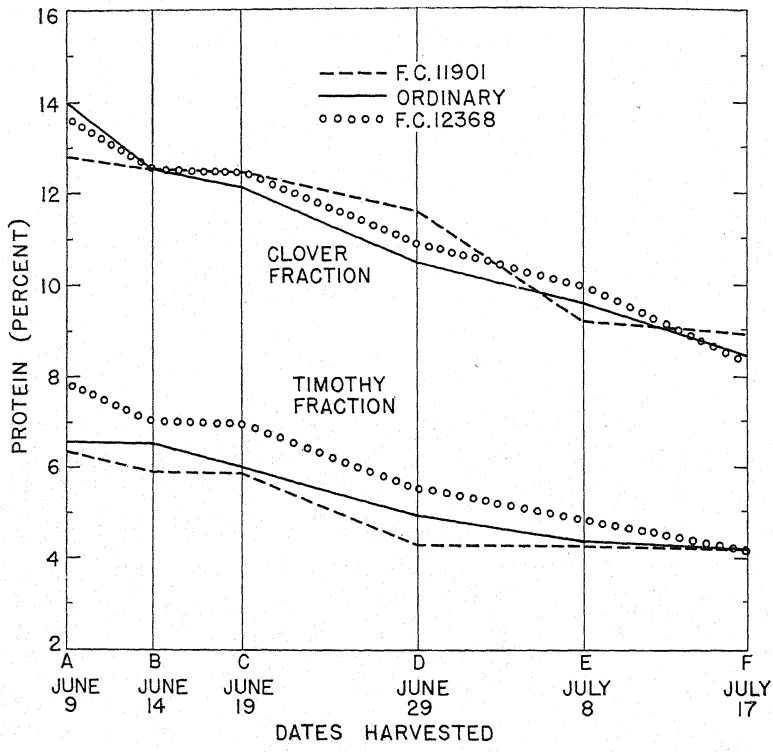


FIGURE 9.—Percentages of protein in the timothy and clover fractions in 1933.

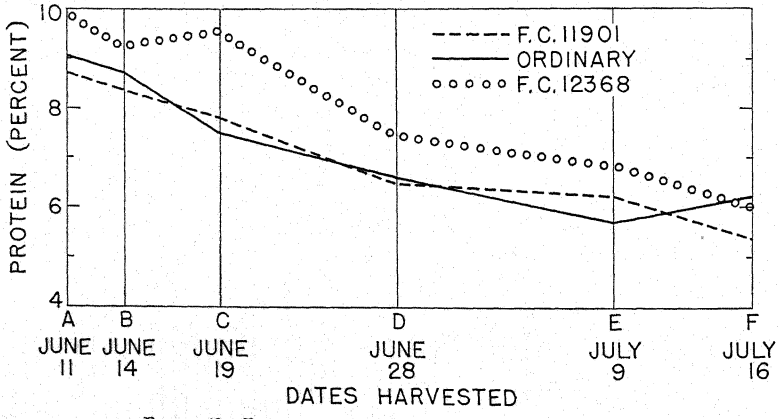


FIGURE 10.—Percentages of protein in the 1934 crop of timothy.

At all times, with minor exceptions, the late timothy, alone or in the mixed hay, contained a higher percentage of protein than the hay produced by the early or medium strains of timothy. The relatively high percentage of protein in the late timothy and clover mixture may be attributed in part to the larger proportion of clover which it contained, as well as to the fact that the percentage of protein was higher in its timothy fraction than in that of the early or medium timothy mixtures.

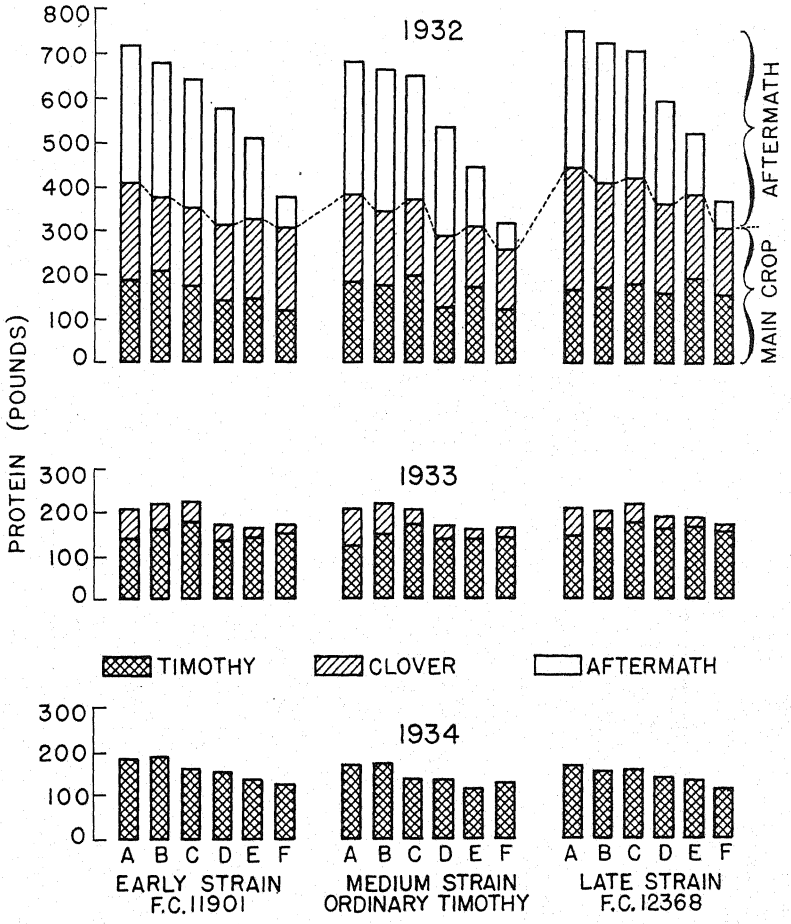


FIGURE 11.—Yields of protein per acre, produced by timothy and by clover, in 1932, 1933, and 1934.

The percentages of protein were higher in the timothy harvested in 1934 than in the timothy fractions in either 1932 or 1933, even though in both of these years the timothy was growing in mixture with clover. A grass grown in mixture with a legume usually contains a greater proportion of protein than one that is grown alone (*8*, *12*). The application of sulphate of ammonia made in the spring of 1934 may explain in part the relatively high percentages of protein in the timothy in that season (*13*). A second contributing factor was probably the

TABLE 5.—*Percentages and yields of protein per acre in the hay harvested in 1932–34*

Year	Crop	Protein per acre at indicated harvest period[1] (main crop) in—																	
		Early timothy (F. C. 11901)						Medium timothy (ordinary variety)						Late timothy (F. C. 12368)					
		A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
1932	Main crop:	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
	Timothy fraction	6.81	6.02	5.40	4.49	4.48	3.57	6.92	6.13	6.30	4.32	4.88	3.57	7.89	7.27	7.04	5.84	5.84	4.37
	Clover fraction	12.41	12.41	11.05	10.81	11.39	10.19	13.45	11.56	11.39	10.58	10.99	10.53	13.45	11.21	11.50	11.21	11.78	9.90
	Total (mixed hay)	8.95	7.87	7.25	6.50	6.71	5.97	9.41	7.94	8.00	6.47	6.51	5.50	10.60	9.11	9.07	7.92	7.73	6.03
	Aftermath: Mixed hay[2]	12.68	13.02	13.02	14.00	15.47	16.86	12.38	12.84	13.37	14.47	14.65	15.70	13.25	13.66	13.37	13.89	15.40	17.21
1933	Main crop (only):																		
	Timothy fraction	6.35	5.88	5.88	4.31	4.31	4.25	6.52	6.52	6.06	4.95	4.43	4.25	7.81	6.99	6.99	5.54	4.84	4.25
	Clover fraction	12.87	12.47	12.47	11.59	9.21	8.97	14.10	12.47	12.17	10.43	9.61	8.51	13.69	12.47	12.47	10.84	9.96	8.39
	Total (mixed hay)	7.50	6.85	6.58	4.95	4.55	4.47	8.25	7.61	6.57	5.59	4.81	4.51	8.94	7.64	7.62	5.98	5.03	4.43
1934	Main crop (only): Timothy	8.79	8.39	7.81	6.56	6.21	5.42	9.18	8.79	7.53	6.61	5.76	6.21	9.93	9.23	9.58	7.42	6.84	5.99
1932	Main crop:[3]	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
	Timothy fraction[4]	186	200	172	141	145	114	177	172	191	123	167	122	170	172	177	162	197	157
	Clover fraction	211	166	171	159	174	186	215	165	170	158	136	138	274	235	240	199	183	151
	Total (mixed hay)	397	366	343	300	319	300	392	337	361	281	303	260	444	407	417	361	380	308
	Aftermath: Mixed hay[2]	306	302	289	265	184	71	297	320	285	251	138	58	307	316	296	237	141	61
1933	Main crop (only):																		
	Timothy fraction[4]	141	158	181	136	145	152	125	153	173	132	136	142	147	164	178	162	174	161
	Clover fraction	63	58	47	35	17	17	80	65	32	38	23	19	63	41	40	30	14	14
	Total (mixed hay)	204	216	228	171	162	169	205	218	205	170	159	161	210	205	218	192	188	175
1934	Main crop (only): Timothy	184	189	160	154	134	124	168	169	139	132	118	129	166	154	159	142	123	111
1932–34	Total	1,091	1,073	1,020	890	799	664	1,062	1,044	990	834	718	608	1,127	1,082	1,090	932	832	655

[1] See table 1 for explanation of harvest periods.
[2] Aftermath harvest all on 1 date, Aug. 31.
[3] Protein in the fractions of the aftermath was not determined.
[4] Includes the small percentage of weeds.

unusually dry season of 1934, preceded by a season with rainfall 25 percent below normal. In years of low rainfall there is likely to be an accumulation of nitrate nitrogen in the soil (*1*). An abundance of nitrate nitrogen in the soil would tend to increase the protein content of the timothy, especially if the growth subsequent to nitrogen assimilation is limited.

In the aftermath produced in 1932 and harvested on August 31, there was a gradual increase in the percentage of protein as the time of harvesting the main crop became later. This was to be expected, since the aftermath growth was relatively younger as the date of harvest for the main crop was advanced.

YIELD OF PROTEIN

The yields of protein attained a maximum at the A period of harvest in the first crop in 1932 and at the A, B, or C period in the crops of 1933 and 1934, but at the D, E, and F periods, even with greater yields of hay, the amount of protein became less (figs. 3 and 11).

In the aftermath of 1932 the percentage of protein increased as the time of harvesting the first crop was delayed, but since the yield of hay in the aftermath was smaller the later the first crop was harvested, the yield of protein in the aftermath was also smaller.

Since clover contains a higher percentage of protein than does timothy, and since the first crop and the aftermath of the first season contained much clover, the yields of protein per acre for the first season were determined largely by the clover fraction of the hay. In the second season's crop the clover fractions were relatively small and so had little influence on the protein content of the hay.

COLOR AND GRADE OF HAY

In 1934, samples of the hay collected at each time of harvest were analyzed for degree of green color and were graded according to the United States hay standards. These records (table 6) were made by the Division of Hay, Feed, and Seed, Bureau of Agricultural Economics, United States Department of Agriculture.

TABLE 6.—*Color analyses and grades of hay produced by early, medium, and late timothy at 6 stages of development in 1934*

Stage [1]	Date of harvest	Strain	Color reading or hue [2]	United States class and grade
A	June 11	F. C. 11901	8.81	U. S. No. 1 Extra Green Timothy.
		Ordinary	9.21	Do.
		F. C. 12368	9.27	Do.
B	June 14	F. C. 11901	8.75	Do.
		Ordinary	8.92	Do.
		F. C. 12368	9.25	Do.
C	June 19	F. C. 11901	8.84	Do.
		Ordinary	8.78	Do.
		F. C. 12368	9.23	Do.
D	June 28	F. C. 11901	6.94	Do.
		Ordinary	6.00	Do.
		F. C. 12368	7.78	Do.
E	July 9	F. C. 11901	4.74	U. S. No. 1 Timothy.
		Ordinary	4.21	U. S. No. 2 Timothy.
		F. C. 12368	5.90	U. S. No. 1 Timothy.
F	July 16	F. C. 11901	2.94	U. S. No. 3 Timothy.
		Ordinary	3.82	U. S. No. 2 Timothy.
		F. C. 12368	4.71	U. S. No. 1 Timothy.

[1] See table 1 for explanation of harvest periods.

[2] As the percentage of green color decreases, the color reading becomes lower. The complete reading for each sample is "8.81y," "9.21y," etc.

Each sample of hay was cured in a barn, where it was protected from sun, dew, and rain. If the hay had been cured in the field in the usual way, sometimes under relatively poor weather conditions, the ratings for color and grade, for some of the samples at least, probably would have been lower.

Grades of hay are based chiefly on the mixture of plants of which it is composed and on color. Since these samples were composed of clear timothy, the differences in the grades may be attributed entirely to the differences in the proportions of green parts.

As the season advanced and the proportions of dry brown leaves, stems, and heads increased, there was a gradual decrease in the color reading or hue of the hay. Because of the favorable conditions under which the samples were cured, all strains were green enough through June 28 to make hay classed as "U. S. No. 1 Extra Green Timothy." After that the grades were lower.

At all periods of harvest, the color reading, or hue, of the late strain was higher than that of either of the other two strains. At the A, B, and F periods of harvest, the medium strain was a little greener than the early strain. At the C, D, and E periods the early strain was slightly greener than the medium strain. This was due largely to the tendency of each leaf blade of the early strain to remain green somewhat longer than the corresponding leaf on a stem of the medium strain (*6*). The relatively low rating of the early strain at the last stage of harvest was in part due to the large number of mature or nearly mature heads.

SUMMARY AND CONCLUSIONS

An early selection of timothy (F. C. 11901), a late selection (F. C. 12368), and ordinary commercial or medium timothy were grown in mixture with medium red clover and harvested at six periods, representing different stages of maturity, for a 3-year period. A main crop and an aftermath of mixed timothy and clover were harvested in 1932; one crop, composed chiefly of timothy, in 1933; and a crop of clear timothy in 1934.

At North Ridgeville, Ohio, the early strain blooms and matures approximately 4 days earlier, and the late strain 7 days later, than ordinary timothy. Although the different harvest periods were not so spaced as to permit exact comparisons, in general the results indicate that the early timothy will produce yields of hay equal to or greater than the yields produced by the medium timothy when the latter arrives at the same stage of development several days later, and that the percentage and yield per acre of protein of the early timothy will equal or exceed that of the medium timothy. Likewise, the late selection harvested at any time will produce yields of hay approximately equal to the yields produced by the medium timothy at the same stage of development about a week earlier and containing a percentage and yield per acre of protein equal to that for the medium timothy.

If the main crops only are considered, the yields of hay increased as the timothy progressed from heading to maturity.

In 3 years, the early selection produced larger yields of hay than ordinary timothy at all periods of harvest. At the three earliest harvest periods the late selection produced less than the medium strain, but at the three latest periods it produced more.

When the aftermath is included in the total yields of hay the advantage of early harvesting, especially for the first season's crop, becomes apparent. Since the aftermath decreases rapidly after the C harvest period, the importance of efficient utilization of the aftermath through early harvesting of the first crop is indicated. The records of yields for the 3-year period—aftermath included—show more advantage in early harvesting for the early timothy than for the late timothy.

The percentage of protein in the hay declined in all three timothy strains and also in the timothy and clover fractions as the date of harvest became later. The late timothy was higher in protein than the medium or early timothy at all comparable dates of harvest. Medium and early timothy differed but little in protein content on comparable dates of harvest.

The yield of protein per acre decreased as harvest was delayed; markedly so after the C period. A tendency was noted for the late timothy to exceed the early and medium timothy in yield of protein per acre. This was due in part to the higher percentage of protein in the late timothy fraction and in part to the larger proportion and yield of clover growing with it in the first year's crop.

The percentage of green color and the United States grades of hay produced by the early and the medium timothy were not greatly different at the different harvest periods. At all times the percentage of green color in the hay produced by the late timothy was higher than in the hay produced by either early or medium timothy, and as the season advanced the grade of the late timothy was higher than that of the other two strains.

In northern latitudes, in rotations in which the meadows are maintained 2 or 3 years, late timothy may be preferable to early or medium timothy when farm practices make late harvesting desirable.

When yields of hay, yields of protein, percentages of protein, and grades of hay are all considered, it becomes evident that harvesting when the variety of timothy is in early bloom, or even before, is preferable to harvesting later.

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RESPIRATION AND OXIDASE AND CATALASE ACTIVITY OF APPLE AND PEAR FRUITS[1]

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INTRODUCTION

In connection with certain investigations of apples and pears in storage the question arose as to the relationship between respiratory intensity and oxidase and catalase activity. A review of the literature showed contradictory evidence. In some cases the correlation was strongly positive, in some negative, and in others oxidase, catalase, or respiration preceded, followed, or acted independently of the others.

Since certain disorders of stored fruit, such as scald, soft scald, and bitter pit of apples and loss of ripening capacity in pears, are physiological in nature and may be correlated with, or caused by, respiratory disturbances during storage, a study of the correlation between respiratory intensity and oxidase and catalase activity of apple and pear fruits was made. Numerous determinations of the rate of respiration and of the oxidase and catalase activity were made at Wenatchee, Wash., during 1933 to 1936, under conditions as thoroughly standardized and controlled as possible.

In its broadest sense respiration is a rather involved process of breaking down respirable materials through various stages to the production of the final products (*40*).[2] However, in the present work the writers have used it in a restricted sense to refer only to the final oxidation of the intermediate substances and have taken the carbon dioxide liberated as a measure of the respiratory intensity. Certain enzymes, including oxidase and catalase, may be involved in respiration, and while "respiratory intensity" and "enzyme activity" may not be mutually exclusive, the writers have separated them for the purpose of comparison, to see whether they are always correlated and react similarly to stimuli. Also the term "oxidase," as used by the writers, refers to those enzymes that oxidize various substances directly without the addition of hydrogen peroxide.

Since fruits are living organisms, the rate of respiration is accepted as a measure of their physiological activity. The enzymes oxidase and catalase are sometimes spoken of as respiratory enzymes and are usually assigned an important role in respiration. However, the part they play is largely hypothetical. In the laboratory the oxidases as prepared by present methods do not oxidize the carbohydrates or fats used in respiration. The role of catalase is even more doubtful. Hydrogen peroxide, the substance known to be attacked by catalase, has not been found in the living tissue, although there is evidence that it is produced there (*44*). The wide distribution of catalase and the close correlation of respiratory intensity and catalase activity, espe-

[1] Received for publication July 21, 1937; issued April 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 384.

cially in some of the earlier work, doubtless was a factor in causing investigators to classify catalase as a respiratory enzyme. Later work has not always yielded such conclusive evidence, and in many cases catalase activity has either preceded, followed, or acted independently of respiratory intensity.

REVIEW OF LITERATURE

The literature dealing with the effect of environmental conditions on respiratory intensity and oxidase and catalase activity is so voluminous that it will be necessary to limit this review to certain papers bearing directly on the interrelations of these three systems. For a more complete discussion and bibliography, the reader is referred to Miller (*26*) or to any of the many other excellent reviews and bibliographies dealing with the subject.

Appleman (*1*), working with potatoes, found that where the tubers were treated with ethyl bromide or removed from storage at 3° C. to room temperature, or greened through exposure to light, the respiration rate and catalase activity were greatly accelerated, while the oxidase activity remained practically constant. He also found greater catalase and respiratory activity in the bud half of McCormick tubers, and no difference in the oxidase activity as measured by the Bunzell method; however, colorimetric results obtained by using aloin as the oxidizable substance gave greater oxidase activity in the stem half. Neither method disclosed any correlation between oxidase activity and respiration. There was a large varietal difference in respiration and oxidase activity, Carmen No. 1 respiring more rapidly than McCormick and possessing only one-fourth the oxidase. The catalase activity in the two varieties was strikingly correlated with respiration. These results led Appleman to conclude that in potato tubers there is no correlation between respiration rate and oxidase activity but a striking correlation between respiration rate and catalase activity. The same author (*2*) later found a close correlation between respiratory intensity and catalase activity in sweet corn in the milk stage. In this stage the respiration rate was very high when the corn was first pulled but fell off rapidly with storage. Catalase activity showed a decline with storage proportional to the decline in respiratory intensity. Appleman's work led him to believe that the catalase activity of the expressed juice from both sweet corn and potato tubers was a fair index of the comparative intensity of respiration in the tissues. He states (*2, p. 209*): "The data from both plant and animal tissues available at present seem to justify the general induction that catalase action is invariably correlated with the oxidative processes involved in respiration."

After treating potato tubers with various chemicals to alter the respiration rate, Miller et al. (*28*) found no direct connection between the respiratory rate and catalase and peroxidase activity. Acceleration of enzyme activity followed the increase in output of carbon dioxide, and it occurred after the peak of respiratory activity. Certain chemicals that increased respiration depressed enzyme activity while the respiration rate was most active, but later the enzymes showed greater activity than in the untreated lot. Alcohol decreased respiration but increased the catalase activity. This chemical had a tendency to lower slightly the peroxidase. These investigators reported

also a close correlation between catalase and peroxidase activity, although in certain cases, notably in the alcohol-treated lots, there was a divergence in activity.

Davis (*10*) concluded that blackheart of potatoes was caused by high respiratory activity and a failure of the gaseous exchange to keep pace with the rate of respiration. The catalase activity of affected tissues was not correlated with respiratory intensity, but was more nearly correlated with the oxygen content of the intercellular spaces.

Rhine (*36*) found a large and immediate rise in respiration during the germination of seeds; catalase activity was first decreased and then accelerated. The early curves of respiration and of catalase activity therefore diverged widely. Rhine concluded that catalase could be used as an indicator of metabolism only in cases where there is no rapid change in respiration.

Lantz (*23*) studied the relation between the rate of respiration and the catalase activity during germination at different temperatures of strains of corn varying in chemical composition. In all strains, at all temperatures, catalase activity decreased in the early stages of germination, then increased to a maximum, and finally decreased again as the reserve food of the grain was used. Respiration increased slowly in the early stages of germination and more rapidly in the later stages. Catalase activity at 30° C. did not increase proportionally with the accelerated metabolism caused by the higher temperature. In most cases catalase content at 30° was less than at 20°, and a temperature of 42° markedly reduced the catalase content of corn seedlings. Respiration rose rapidly with an increase in temperature, but catalase failed to show a corresponding increase. Lantz found some correlation between catalase activity and rate of respiration during germination of the different strains of corn at 20°, but no correlation was apparent at 10° and 30°. In general, his results failed to show a close correlation between catalase activity and respiration rate, and he concluded that the evidence did not justify the theory that catalase is the enzyme chiefly concerned in physiological oxidation, but more nearly supports the theory that catalase prevents excessive oxidation.

Becht (*5*) found the blood of animals of the same species, under identical conditions, to vary from 100 to 1,000 percent in catalase activity. He considered catalase activity of doubtful importance in the oxidation process. Morgulis (*29*) found that exposure of frogs to temperatures that affected the metabolic rate from 300 to 400 percent had no influence on the catalase content, and he concluded that whatever the function of catalase might be in the organism it certainly was not a measure of metabolic activity. Seymour (*38*) determined the catalase content of the ventricular muscle of turtle hearts. Hearts with a rapid rate as a result of warming (range 1.5 to 40 times the controls) varied in catalase content both above and below the controls, the greater number showing less catalase.

Bunzell and Kenyon (*7*), working with the growing potato plant, found oxidase activity greater in the tuber than in the foliage of the same plant while the catalase activity was constantly two to four times greater in the foliage. The oxidase activity of the foliage was greatest in the early stages of development, declined with growth, and rose again when growth had ceased, whereas the catalase activity

in each instance behaved in just the opposite way. These investigators suggested a reciprocal relationship between oxidase and catalase activity.

Reed (*35*) concluded that the substances affecting the decomposition of hydrogen peroxide (catalase) are not of necessity concerned with enzymes that accelerate peroxide oxidations (peroxidases or oxidases) in pineapples. He reported no catalase activity in green pineapples and concluded that catalase is not universally present in living cells as Loew and others supposed. Harvey (*20*) also failed to find any catalase in blue-green algae growing in hot springs at a temperature of 65° to 73° C.

Tyson (*43*) has shown catalase activity to be positively correlated with vigor of growth and size of plant in sugar beets and the oxidase activity to be greater in those plants in which the growth was inhibited. Ezell and Crist (*14*) found that the correlation between the activity of oxidase and growth or size of plants was slight, with a tendency to be negative in lettuce, radish, and spinach plants. The correlation of growth or size of plants with catalase activity was better than with oxidase activity and significantly negative.

Gustafson et al. (*16*), working with tomato fruits, concluded that "respiration, growth and catalase activity go hand in hand," although in one set of experiments tomatoes growing more rapidly than 4 percent per day gave less catalase activity than those growing between 2 and 4 percent. Haber (*18*), also working with tomato fruits, studied the relationship of soil reaction, growth, and catalase activity. He used soils varying in pH values from 9 to 4 and found that the neutral soil gave the greatest yield and the lowest catalase activity. Soil reaction had no effect on oxidase activity. Oxidase activity was greater in ripe fruits than in green ones, and catalase activity was greater in green mature fruits than in ripe ones.

De Villiers (*11*) showed an increased catalase activity with increasing maturity in grapes, but when the grapes became fully ripe there was a slight decrease. Oxidase activity and respiration rate decreased with maturity, the curves being almost parallel.

Johnson (*22*) reported the effect of X-rays on *Helianthus annuus*. She found that irradiation of seeds or seedlings caused a decrease in rate of growth, in catalase activity, and in carbon dioxide production (respiration). Oxidase activity was unaffected by irradiation even in dosages heavy enough to cause seedlings from irradiated seeds to die after reaching a height of 1 to 2 cm. Normal and abnormal leaves gave the same oxidase activity.

In work with diseased plants Bunzell (*6*) found that the diseased material had a greater oxidase activity than healthy material. The characteristic symptom of the diseases studied (spinach blight, mosaic of tobacco, leaf curl of potatoes, curly dwarf of potatoes, and curly top of sugar beets) was a stunting of plant growth.

Dexter (*12*) studied the rate of respiration and the catalase and oxidase activity of cabbage and of winter wheat plants during exposure to low temperature (2° C.). He found that the respiration rate of all samples continuously decreased during storage at this temperature, while the oxidase activity appeared unchanged. In cabbage the catalase activity remained unchanged either in the light or in the dark (for 5 days), but in winter wheat it increased in the dark and decreased in the light.

Pope (*33*) reported a negative correlation between respiration rate and catalase activity in homologous younger leaves of comparable barley plants, but since this was contrary to the results of other investigators, he concluded that it was highly probable that any correlation was fortuitous.

Morinaga (*30*) showed that dry seeds of rice contained about one-tenth as much catalase as wheat, barley, oats, or rye. Rice germinating aerobically contained about seven-tenths as much catalase as germinating wheat, oats, or barley. Catalase did not increase in the course of anaerobic germination, but in a medium of reduced oxygen it increased slowly. Hence he concluded that the rate of increase of catalase activity was a function of the free oxygen in the medium. Aerobically grown seedlings with high catalase used much more oxygen than anaerobically grown seedlings with low catalase activity. The anaerobic seedlings gave off a comparatively large amount of carbon dioxide during respiration, whether under normal aerobic or under anaerobic conditions.

Ransom (*34*) found that the catalase activity of water-saturated fruits and seeds of Polygonaceae was greatest at 3° to 9° C. and lowest at 30°, while the rate of respiration was lowest at 3° to 9° and highest at 30°. However, at a given temperature catalase activity and respiration rate were found to be correlated with the rate of afterripening and germination.

Heinicke (*21*) found no consistent correlation between catalase activity and respiration rate in dormant excised apple twigs.

Harding (*19*) found no correlation between catalase activity and respiration rate at 30° and 36° F. in Grimes Golden apples, but at 50° there was a correlation. He reported: "Catalase activity is independent of, and unrelated to temperature." (Respiration rate is usually considered to be directly related to temperature.) "Oxidase and catalase activity were independent of each other, and oxidase was not found to be significant as an indicator in denoting the development of soggy breakdown." Under cold-storage conditions, an increase in catalase activity was a fairly accurate indication of the approach of soggy break-down. Neller (*31*) noted a tendency to a higher catalase content in Jonathan apples going through the breakdown process, and lower activity in the advanced stages of breakdown. In normal fruits that did not develop break-down, the catalase activity increased during early storage and decreased late in storage life. Neller's findings tend to corroborate the theory that catalase may be used as an index of metabolic activity, physiological break-down being associated with, or caused by, an accelerated metabolic rate.

Drain (*13*) reported that the Oldenburg apple was low in catalase as compared with the Winesap apple, although at 25° C. the Oldenburg variety respired about twice as fast as Winesap at the same temperature. Winesap apples held at 25° increased in catalase activity over similar lots held at 0°, but the respiration rate remained practically the same. A lower respiration rate and less oxidase activity were noted in hard ripe fruit than in ripe fruit. Drain concluded that respiration rate and catalase activity were not closely correlated in apple varieties.

Magness and Burroughs (*25, pp. 79–85*) determined the respiration rate and catalase activity of apples after removal from cold storage to

a higher temperature, and found a gradual increase in catalase activity throughout the duration of the test (17 days). Respiration reached a maximum in approximately 1 day. They concluded that catalase followed respiration and was associated with the total respiration that had taken place rather than with the rate at any particular time. Cellar-stored Winesap apples high in catalase had a lower respiration rate when transferred to 65° F. than did fruit from 32° storage low in catalase. Baldwin apples removed from 65° storage, where high catalase activity had developed, showed no decrease in catalase activity after 2 months' storage at 32°, indicating that catalase was not directly related to temperature. Later Magness and Ballard (*24*) found that the highest point in catalase activity came sometime prior to the period of greatest respiratory activity in Bartlett pears stored at 60°. When the pears were almost full ripe, the respiratory activity was increasing while the catalase was decreasing rather rapidly.

Carrick (*8*) found that freezing Winesap apples at −7.5° to −8.5° C. for 3 to 6 hours caused their respiration rate at 0° to increase as much as 85 percent above their previous normal performance at that temperature. This increased rate lasted for several days. With McIntosh apples 4 days after freezing, he found (*9*) the catalase activity lower in the 3-hour treatment but somewhat higher in the 6-hour treatment. With Baldwin apples 3 days after freezing, those frozen for 3 hours did not vary from the controls in catalase activity; those frozen 6 hours showed a slight increase. Seven days after freezing, both the 3- and the 6-hour treated fruits showed a slight increase in catalase activity.

The available experimental evidence is not such as to give conclusive proof that rate of respiration is dependent upon oxidase or catalase activity as measured by present methods. Rather, it raises serious doubt as to whether these activities are directly correlated with the rate of respiration.

MATERIALS AND METHODS

Fruits used in these studies were selected in the orchard for uniformity of growing conditions, size, and other external factors, thereby insuring comparable material for the various phases under investigation. After harvest all lots were treated uniformly except for those variations being studied. When cold storage was used the fruit was stored within a few hours after harvest, and in all cases respiration lots and lots used for enzyme determinations were given comparable treatments.

Rate of respiration was usually determined by enclosing fruit in an airtight container, aspirating with air free from carbon dioxide, and absorbing the respired carbon dioxide in barium hydroxide in Truog absorption towers. Exceptions to this procedure are noted below. Oxidase activity was determined iodometrically as described by Guthrie (*17*). A uniform reaction temperature was obtained by drawing the air through a constant-temperature bath held at 25° C. Enzyme determinations were made in duplicate and if not in close agreement were discarded and samples re-run. Catalase activity was determined by a water-displacement method similar to that described by Heinicke (*21*). Uniform shaking was accomplished by means of a mechanical shaker run by an electric motor geared so that the solution flowed 108 times per minute from one end of the tube to the other.

An even temperature was obtained by immersing the tubes in a thermostatically controlled bath held at 25°. Preliminary tests showed this temperature to give nearly maximum activity with the materials studied.

Balls and Hale (*4*) reported that catalase may exist in the inactive state. Activation of this inactive form could be accomplished by the addition of an activator, such as Kolmer's antigen or an extract prepared from heart tissue, as shown by Swenson.[3] Catalase determinations were made on various samples to which the above-mentioned activators were added. Representative data are given in table 1. The activity reported represents 1 cc of juice plus 1 cc of the activator diluted as indicated.

TABLE 1.—*Effect of activators on catalase in juice from pears*

Activator	Dilution	Activity [1] (O_2 liberated)	Activator	Dilution	Activity [1] (O_2 liberated)	Activator	Dilution	Activity [1] (O_2 liberated)
		Cc			*Cc*			*Cc*
Blank (water)		10.6	Blank (water)		14.7	Heart	0	12.6
Antigen	1:100	10.0	Antigen	0	3.3	Do	1:100	13.4
Do	1:500	10.5	Do	1:100	13.2	Heart (no pear juice).	0	.4
Do	1:1,000	9.8	Do	1:500	13.1			

[1] Activity of 1 cc of juice plus 1 cc of activator diluted as indicated.

Table 1 shows that the activators did not increase the catalase activity of the fruit juice. This is not surprising, since the juice used had been pressed from the tissue. Swenson and James (*41*) reported inability to separate the inactive catalase from the living cell.

Several methods of taking samples for enzyme determinations have been reported. Most of these include the use of calcium carbonate or other materials to neutralize the acids present in the tissue and thus prevent the destruction of the catalase. In a study of this kind it is of the utmost importance to have a uniform method of taking samples and determining the enzyme activity, while the particular method employed may be of relatively less importance. The methods employed here were adopted for convenience and represent those that would give easily readable differences. Calcium carbonate was used in the preparation of catalase samples, since unneutralized juice gave a very low reading. Rose et al. (*37*) have shown that ions other than hydrogen or hydroxyl may be important in regulating oxidase activity, and that in neutralizing the acids the effect of the salts formed must be taken into account. Since a satisfactory reading could be obtained with the unneutralized juice, no carbonate or other chemicals were added in the preparation of the oxidase samples.

In taking samples for enzyme analyses the fruit was cut through the greatest transverse diameter, thus dividing the seed cavity in half. The seeds were removed and a portion approximately 1 cm thick was taken from each of the cut surfaces, giving a disk from the greatest transverse diameter of the fruit. Ten or more fruits were used for each sample. Sections were taken from the calyx half for catalase measurements. The sections were weighed and 1 percent of calcium carbonate was sprinkled over the cut surfaces. The tissue was then ground in a food chopper with a nut-butter attachment, and

[3] SWENSON, T. L. ROLE OF CATALASE IN BIOLOGICAL OXIDATION AS DEMONSTRATED BY PROTEUS VULGARIS. Thesis, Amer. Univ. (On file in Amer. Univ. Libr., Graduate School.) 1933.

the juice was squeezed through two thicknesses of cheesecloth. It was then filtered through double cheesecloth without pressure, to remove the tissue forced through at the first filtering. The juice was held in the refrigerator at 40° F. for 24 hours before determining the catalase activity. For each determination 1 cc of the neutralized pear juice and 5 cc of dioxygen were used. Since apples are relatively weak in catalase, 5 cc of juice and 5 cc of dioxygen were used. Oxidase samples taken from the stem half were ground, and the juice was extracted as for catalase. Samples were prepared in the forenoon, and enzyme determinations were made in the afternoon usually after the lapse of about 3 hours. The oxidase activity varied widely under the various conditions, and it became necessary to alter the amount of sample in order to insure an excess of oxidizable substance in the substrate. However, the amount of juice used was the same throughout any particular experiment and varied only between experiments.

In presenting the results, respiration is reported as milligrams of carbon dioxide per kilogram-hour. Oxidase is reported as cubic centimeters of N/100 sodium thiosulphate, and catalase as cubic centimeters of oxygen liberated in 5 minutes.

EXPERIMENTAL WORK

RESPIRATION RATE AND ENZYME ACTIVITY OF DELICIOUS APPLES AS INFLUENCED BY EXTERNAL CONDITIONS

The apples used in this experiment were harvested at optimum maturity and stored immediately at 32° F. until ready for use in January. Since certain chemicals are known to affect the rate of respiration in potatoes and other plants, it was decided to treat apples with some of these chemicals and determine the effect on the rate of respiration and the enzyme activity. The substances selected were ethylene and ethylene chlorohydrin to increase respiration and ethyl alcohol to lower it. Preliminary tests showed that when fruit was removed from 32° and placed at 65° the increase in rate of respiration due to the change in temperature alone was so great that differences due to the chemicals were of doubtful significance. Representative rates at 65° were as follows:

Check untreated	19. 9 mg CO_2 per kilogram-hour
Ethyl alcohol	18. 9 mg CO_2 per kilogram-hour
Ethylene	21. 1 mg CO_2 per kilogram-hour
Ethylene chlorohydrin	21. 1 mg CO_2 per kilogram-hour

Since the respiration differences were small, no enzyme determinations were made on these lots.

In the tests reported below the apples were treated at 32° F. for 30 hours as follows:

Lot 1.—Check, aerated continuously.

Lot 2.—Aerated with air that had bubbled through 95-percent ethyl alcohol before entering respiration chamber.

Lot 3.—Ethylene, 1 part to 1,000 parts of air, in a closed system with potassium hydroxide as absorbent of carbon dioxide.

Lot 4.—Aerated with air that had bubbled through 40-percent ethylene chlorohydrin before entering respiration chamber.

The results are given in figure 1. The rates of respiration are the average for 95 hours following the beginning of treatment and are representative of differences noted at intervals during the treatment. Enzyme samples were taken at the end of the 95-hour period.

The evidence indicates that the respiration rate is not directly cor-

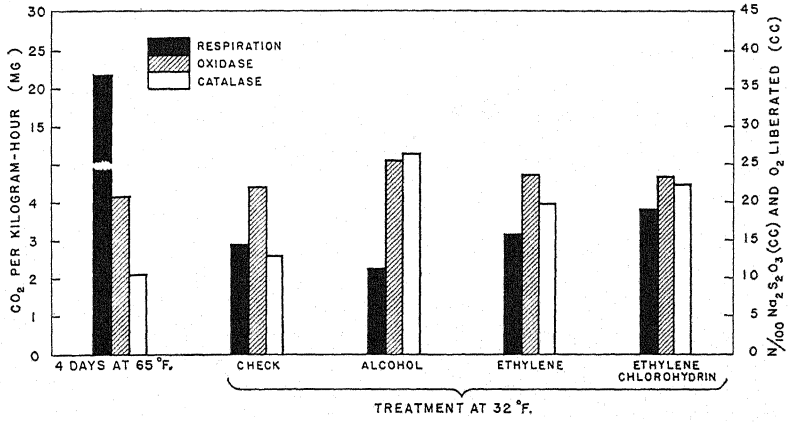


FIGURE 1.—Respiration rate and enzyme activity of Delicious apples as influenced by external conditions.

related with oxidase or catalase activity in cold-stored Delicious apples. The catalase and oxidase also acted independently of each other. The rate of respiration was not affected as much by the chemicals as has been previously reported with potatoes; however, alcohol caused a depressing effect and ethylene and ethylene chlorohydrin an accelerating effect. Both oxidase and catalase activity were greater in the chemically treated fruit than in the untreated check. Catalase activity was from 50 to 100 percent greater in the treated lots, irrespective of whether the respiration rate was increased or depressed. Alcohol, which had a depressing effect on respiration, caused a greater increase in enzyme activity than did ethylene or ethylene chlorohydrin.

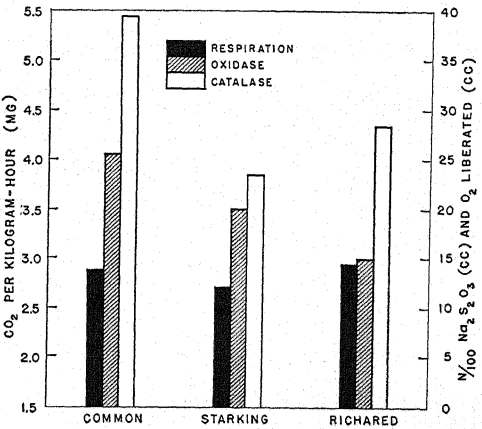


FIGURE 2.—Respiration rate and enzyme activity of different strains of Delicious apples.

Upon the termination of these experiments the check lot of fruit was removed from 32° to 65° F. for 4 days; the respiratory intensity increased 676 percent, while the oxidase and catalase activity decreased slightly. At the end of 8 days at 65° the oxidase activity was still lower, but the catalase activity had more than doubled.

RESPIRATION RATE AND ENZYME ACTIVITY OF STRAINS OF DELICIOUS APPLES

Determinations of rates of respiration and of oxidase and catalase activity were made on three strains of Delicious apples (common Delicious, Starking, and Richared). The fruits were stored at 32° F. immediately after harvest, and determinations were made 36 hours later. The results are given in figure 2.

There is no correlation evident between oxidase and catalase activity and rate of respiration in different strains of Delicious apples. Oxidase and catalase also act independently of each other.

RESPIRATION RATE AND ENZYME ACTIVITY OF ROME BEAUTY AND WINESAP APPLES AS INFLUENCED BY TEMPERATURE

Respiration rate and oxidase and catalase activity were determined on Rome Beauty and Winesap apples at harvest and after storage at 65° and at 32° F. The results are given in figure 3.

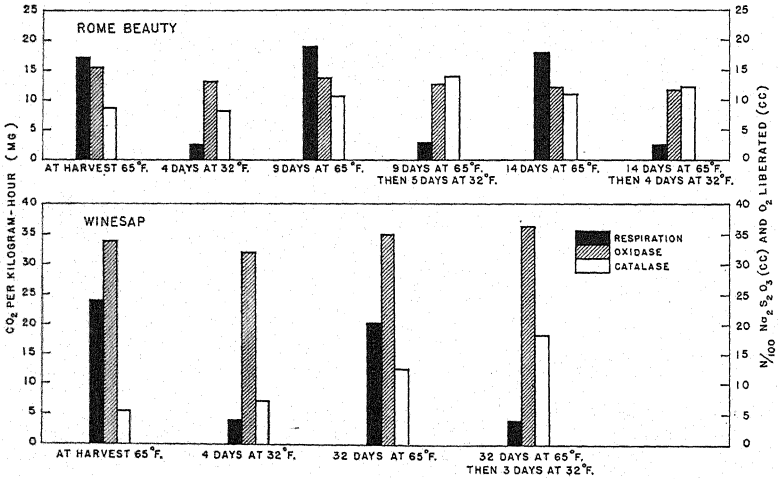


FIGURE 3.—Respiration rate and enzyme activity of Rome Beauty and Winesap apples as influenced by temperature.

With Rome Beauty apples under the different storage treatments the respiration rate ranged from 2.45 to 18.90 mg of carbon dioxide per kilogram-hour, an increase of 671 percent. The oxidase activity ranged from 11.5 to 15.6 cc, an increase of 35 percent. The respiration rate rose during storage at 65° F., reaching a climax in 9 days. Oxidase activity declined slowly but steadily at this temperature. At 32° the respiration rate dropped to approximately 15 percent of its intensity at 65°. There was only a slight decrease in oxidase activity, while in certain cases catalase was greater. In Winesap apples the respiration rate and oxidase and catalase activity showed a similar tendency to act independently of each other.

RESPIRATION RATE AND ENZYME ACTIVITY OF ROME BEAUTY APPLES AS INFLUENCED BY RESPIRATORY STIMULANTS FOLLOWING HARVEST

It was thought that apples treated immediately after harvest while in a preclimacteric respiratory stage might be more subject to respira-

tory stimulants than lots that had been stored at 32° F. until they reached the postclimacteric stage and had then been brought to the warm room. In the latter case it had been found that changes due to the sudden rise in temperature from 32° to 65° largely obliterated the effect of the stimulants. Rome Beauty apples were brought to the 65° room immediately after harvest and subjected to the following treatments:

Lot 1.—Check, aerated continuously.
Lot 2.—Aerated with air that had bubbled through 40-percent ethylene chlorohydrin before entering respiration chamber.
Lot 3.—Aerated with air mixed 50–50 with oxygen gas before entering respiration chamber.

In order to determine the cumulative effect of the gases, the treatment was continued for 12 days and was followed by 5 days' aeration without the gases. The results are given in table 2.

TABLE 2.—*Respiration rate and enzyme activity of Rome Beauty apples as influenced by respiratory stimulants immediately following harvest*

Storage at 65° F. (days)	Check			Ethylene chlorhydrin [1]			Oxygen [1]		
	Respiration rate (CO^2 per kg-hr.)	Oxidase activity (N/100 $Na_2S_2O_3$)	Catalase activity (O_2 liberated)	Respiration rate (CO_2 per kg-hr.)	Oxidase activity (N/100 $Na_2S_2O_3$)	Catalase activity (O_2 liberated)	Respiration rate (CO_2 per kg-hr.)	Oxidase activity (N/100 $Na_2S_2O_3$)	Catalase activity (O_2 liberated)
	Mg	*Cc*	*Cc*	*Mg*	*Cc*	*Cc*	*Mg*	*Cc*	*Cc*
1	14.05			20.25			15.84		
2	16.06			21.63			20.57		
3	17.01	9.2	8.1	21.11	7.2	6.7	20.98	6.8	7.8
4	18.36			21.45			22.69		
5	19.52			21.67			23.37		
6							23.07		
7	18.94			20.87			21.74		
8	18.15								
9	18.22			20.56			21.11		
11	18.68			20.87			21.46		
12		5.6	12.9		6.1	6.8		6.6	13.2
14				19.80			18.12		
16	16.37			21.39			18.23		
17	17.91			22.41			18.82		
18		6.5	12.2		6.4	6.3		6.5	12.7
19	15.82								

[1] Treatment discontinued after 12 days.

While the gases caused an appreciable increase in respiration, the rate of respiration and oxidase and catalase activity were not parallel in Rome Beauty apples subjected to respiratory stimulants immediately after harvest. Ethylene chlorohydrin caused a rapid rise in respiration, which continued beyond the period of treatment. It is possible that the treatment was too severe for the best results. The respiration rate rose rapidly and remained practically stationary for several days; it decreased slightly and then rose above the previous maximum after the treatment was discontinued. Ethylene chlorohydrin could be tasted in the fruit for several days. No visible injury was evident at the end of the treatment, but 6 days later, when the last enzyme determinations were made, approximately one-third of the fruits were showing injury at the core. It is evident that the vapors had been interfering with the catalase activity much earlier, possibly within 3 days.

The apples responded a little more slowly to oxygen gas than to ethylene chlorohydrin vapor. Apparently the increased concentration of oxygen did not interfere with the normal metabolism of the fruit as much as did chlorohydrin. The rate of respiration was higher in the oxygen-treated lot than in the check lot, but otherwise the behavior was much the same. Both lots reached the respiratory climax in 5 days. At the end of 3 days the oxidase activity was lower in the treated lot, but it was approximately the same in both lots at the end of the treatment and again 6 days later. Catalase activity of the two lots was closely parallel during the test.

RESPIRATION RATE AND ENZYME ACTIVITY OF BARTLETT PEARS

Since it is known that very immature fruit respires much more rapidly than mature fruit and also that oxidase and catalase activity are greater in immature fruit, a study was made of the changes in these activities in Bartlett pears during the growing season. The first determinations were made June 11, when the average weight per fruit was 19.1 g, and at intervals thereafter until September 9, when the average weight per fruit was 270.5 g. The last sampling was far past the normal harvest season. The fruit was picked between 8 and 9 a.m. each time throughout the season at several points in the same tree, so as to get a representative sample. The tree was located on a moderately heavy clay loam, was in vigorous growing condition, and bore a good crop of fruit. Immediately after harvest the fruit was taken to the laboratory and the samples were prepared. Fruit used for determining respiratory intensity was placed in airtight containers, and determinations were made in the afternoon of the day the fruit was picked. Oxidase and catalase samples were prepared from comparable lots of fruit in the forenoon of the same day. The oxidase activity was so great in the very immature fruit that it was necessary to reduce the amount of juice taken for analysis from 10 cc, as previously reported, to 1 cc. Pear fruits in general are much richer in catalase than apple fruits, and 1 cc of juice was also used for the catalase determinations. The results are given in figure 4.

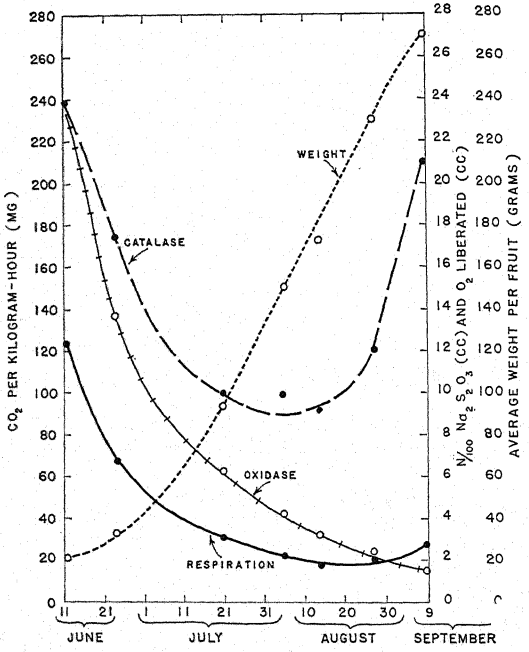


FIGURE 4.—Respiration rate and enzyme activity of Bartlett pears during the growing season.

Figure 4 shows a consistent increase in the size of the fruit throughout the growing season. The rate of respiration and oxidase and catalase activity showed a corresponding decrease until August 14, at which time the fruit showed a pressure test of 17.9 pounds, which is usually recommended for the harvesting of Bartlett pears for best dessert and storage quality. Oxidase activity continued to decrease as long as the fruit remained on the tree, but the rate of respiration and catalase activity increased after the August 14 minimum. If determinations had been made at shorter intervals immediately preceding and following this date a lower minimum might have been recorded, but it is of interest to note that the minimum in both respiration and catalase activity came at the time when experience had showed that the fruit should be harvested. A strong positive correlation is evident between the rate of respiration and catalase throughout the growing season. Oxidase activity also followed closely until the fruit was ready for harvest, but if the fruit was allowed to remain on the tree until it was "tree ripe" the oxidase activity decreased further while catalase activity and the rate of respiration increased.

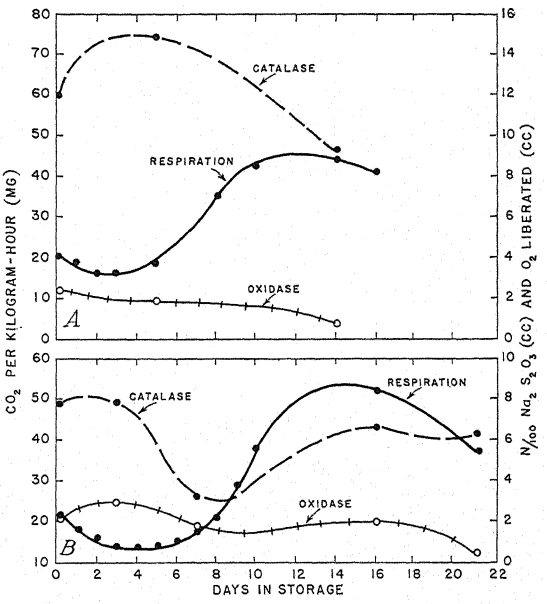


FIGURE 5.—Respiration rate and enzyme activity of Bartlett pears during ripening at 65° F.: *A*, Mature fruit; *B*, immature fruit.

This correlation between rate of respiration and enzyme activity was evident only at harvest. When fruit was held in storage for several days the rate of respiration and enzyme activity diverged. This divergence, shown in figures 5 and 6, held true whether the fruit was harvested while still immature or was permitted to reach full maturity.

In connection with the rise in respiration on removal of the fruit from 32° to 65° F., as shown in figure 6, it should be noted that the rate at the end of the first day at the higher temperature was greater than at the end of the second day. Willaman and Brown (*45*) attribute this rise to a lower carbon dioxide solubility in the cell sap at the higher temperature, with consequent release of the excess gas.

RESPIRATION RATE AND ENZYME ACTIVITY OF ANJOU PEARS DURING STORAGE

Anjou pears were harvested at optimum maturity (11.6 pounds pressure) and stored immediately at 32° F. The rate of respiration and enzyme activity were followed during the storage season. The results are given in figure 7.

It is evident that the rate of respiration is not correlated with enzyme activity in Anjou pears stored at 32° F. The rate of respiration fluctuated during the storage season, but no definite trend was noticeable. Oxidase activity increased steadily, more than doubling during the season. Catalase activity also increased but fluctuated more widely than oxidase. Other varieties gave similar results. Unpublished data have shown that within the commercial picking range the maturity of the fruit at harvest exerts little influence on

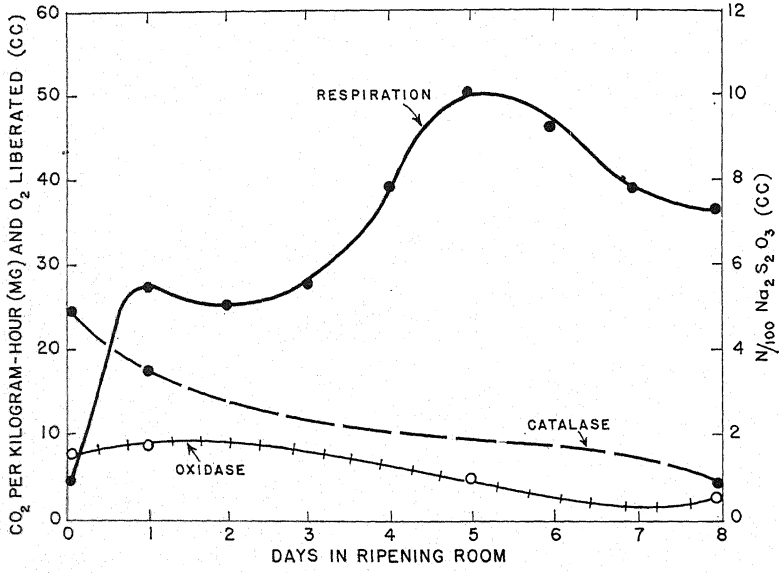


FIGURE 6.—Respiration rate and enzyme activity of Bartlett pears stored at 32° F. for 14 days and ripened at 65°.

the rate of respiration at 32° but exerts considerable influence on the oxidase activity. With the less mature fruit the oxidase activity increases rapidly with time in storage, but with more mature fruit the increase is less.

RESPIRATION RATE AND ENZYME ACTIVITY OF BOSC PEARS FROM DIFFERENT LOCALITIES

Bosc pears grown at Wenatchee and Yakima, Wash., and at Medford, Oreg., were harvested and stored at 30° and 36° F. within 24 hours after harvest. Rates of respiration and oxidase and catalase activity were determined after 3 months' storage. The results are given in figure 8.

The rates of respiration were practically the same in the fruit from the three districts. The averages of the enzyme values from the two temperatures show that the Medford fruit contained about five and one-half times as much oxidase as the Wenatchee fruit and over three times as much as the Yakima fruit. Medford Bosc pears also contained more than twice as much catalase as Wenatchee Bosc and nearly twice as much as Yakima Bosc. At 30° F. the average output of carbon dioxide was about two-thirds of that at 36°. The oxidase activity did not vary greatly, and the catalase activity at 30° was approximately two-thirds of that at the higher temperature.

DISCUSSION

Throughout the work it was evident that the chemical stimulants used were not so effective with apples and pears as has been reported with potatoes. Miller and Denny (*27*) have shown that the amount of ethylene chlorhydrin taken up by potato tubers varies with the permeability of the skin. The skin of apple fruits is comparatively tough and has a coating of natural wax. This skin doubtless is

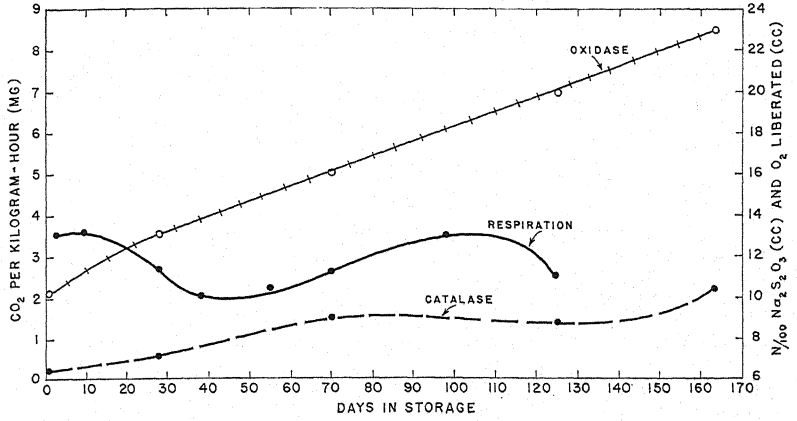


FIGURE 7.—Respiration rate and enzyme activity of Anjou pears during storage at 32° F.

much less permeable than the least permeable potato skin. Pear skins are less tough than apple skins and comparatively free from wax. Gerhardt and Ezell (*15*) have shown that pears absorb gas much more readily than apples, as measured by the internal concentrations of carbon dioxide after the fruit has been subjected to the gas for a definite period. On removal to normal atmospheres

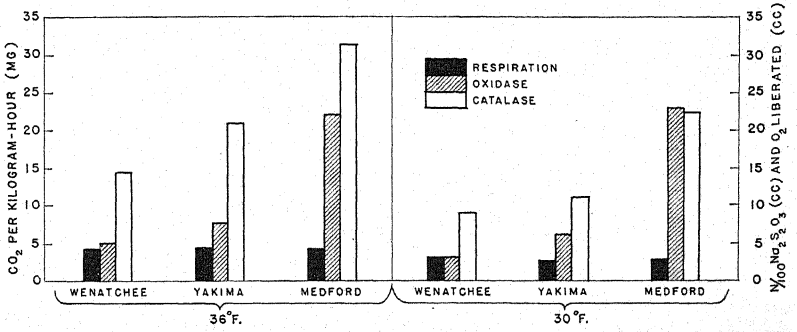


FIGURE 8.—Respiration rate and enzyme activity of Bosc pears from the Wenatchee, Yakima, and Medford districts, stored at 30° and 36° F.

pears also lost the gas more rapidly and reached equilibrium at about the same time as apples, indicating that pears are more permeable to gases than are apples. However, Anjou pears held at 32° F. for 2 months and then treated with ethylene and ethylene chlorohydrin for 48 hours at 32° showed no significant increase in respiration over the check. When treated at 65° for 24 hours no significant differences were noted during treatment or during subsequent ripening.

From the results reported herein it appears that apple and pear fruits are much less susceptible to chemical respiratory stimulants than are potatoes. However, it is well to remember that they represent entirely different types of tissue. Mature fruits, exclusive of seeds, consist of mature cells and are incapable of further growth or division. Potatoes are fleshy underground stems composed largely of storage cells and meristematic tissue in the resting stage. Under favorable conditions they are capable of growth, production of new cells, and the completion of the life cycle, all of which necessitate increased respiration.

The results herein presented show that the intensity of respiration and the activity of the so-called respiratory enzymes, oxidase and catalase, are apparently not closely correlated in apple and pear fruits when held in storage. The data show that the enzymes act independently of the rate of respiration and independently of each other. However, in Bartlett pears sampled immediately after picking there was a close correlation between respiration and catalase activity throughout the growing season. Oxidase activity likewise was closely correlated with respiration until the fruit was ready for harvest, but if the fruit was allowed to remain until "tree-ripe", the oxidase activity decreased while the rate of respiration and catalase activity increased.

Since the status of oxidase and catalase as respiratory enzymes is largely hypothetical, with little or no direct evidence to support the theory that they are responsible for respiratory intensity, and since results of previous work show such wide divergence, one might be inclined to question the part that these enzymes play in respiration. Perhaps the correlations found have been fortuitous and without special significance.

Another interpretation, and one that seems more logical, is to accept oxidase and catalase as respiratory enzymes and credit them with accelerating certain chemical changes that take place during respiration. Certainly it is necessary to assume the presence of some activating substance in the living cell to account for the ease and rapidity with which chemical reactions take place and compounds are broken down to release energy for the cell. However, it has been pointed out (*14, p. 21*) that—

> there is no evidence to support any theory which would embody the concept of there being an exact quantitative balance between the necessary amount of energy and the activity of energy-producing agencies. This would attribute to plant organisms a degree of economy and an inherent capacity for conservation of energy which they may or may not and most likely do not possess.

The above concept of the interrelationship between respiration and oxidase and catalase activity need not conflict with the findings of other workers. Instead it offers a possible explanation of some apparent discrepancies. Oxidase and catalase enzymes could undoubtedly be present in excess in tissues and bear a relation to respiration comparable to that of the large reserves of food materials, none of which would be likely to become limiting under normal conditions. Seeds containing large reserves of stored food respire very slowly until they start to germinate, and the seedlings increase in respiratory intensity to a maximum and then decline; the curve of this process is practically identical with the grand curve of growth (*32, p. 217*). Thus the low points in the respiration curve are at the beginning and

at the end of the life of the plant, at which times the amount of stored food is at its maximum. Miller discusses the effect of food supply on the rate of respiration, and states (*26, p. 733*): "The total quantity of carbohydrate in the cell will thus not influence the rate of respiration except to determine the length of time that the process may continue therein." More recently Appleman and Smith (*3*) have shown that there is no direct correlation between the rate of respiration and the total or reducing sugar content. Possibly nature is not so economical with respiratory enzymes that there would be a supply sufficient to take care of immediate needs only, when there is normally an abundance of reserve materials present in excess of the amount consumed in respiration.

If the foregoing hypothesis be true and the intensity of respiration increases rapidly, as in germinating seed, the enzymes present are sufficient to take care of the increase in respiration, although if the enzyme content is relatively small it may decrease until an additional amount can be produced; this would explain the delayed rise in catalase activity as reported by Rhine (*36*), Lantz (*23*), and Miller et al. (*28*). If the supply of the enzyme is relatively large, as in fruits, then the enzyme activity may increase slowly as additional catalase is produced; and the enzyme activity may follow the rate of respiration, as reported by Magness and Burroughs (*25*), or decrease if conditions are unfavorable for production of the enzyme, as reported by De Villiers (*11*).

That the catalase should increase as the fruit ripens on the tree (fig. 4) but decrease as it ripens in the ripening room (figs. 5 and 6) may seem rather contradictory on first thought. Two explanations may be offered.

(1) The type of ripening is different in the two cases. Bartlett pears harvested at commercial maturity and ripened at 65° F. become soft and juicy with almost a buttery texture. The fruit becomes increasingly soft as it approaches the overripe stage, and the tissue throughout finally collapses. However, fruit allowed to ripen on the tree remains firm and crisp and the texture becomes granular. As maturity advances break-down begins at the core and progresses outward, leaving only a shell of apparently sound tissue filled with broken-down cells, the shell finally collapsing. In these two types of ripening, apparently dependent on different factors during the ripening process, the need for catalase may not be the same.

(2) When fruit is harvested at commercial maturity and ripened at 65° F., all the processes of ripening are katabolic. The products of decomposition may accumulate rapidly to injurious proportions and destroy the catalase. Fruit previously stored at 32° ripens quickly, decomposition products accumulate rapidly, and a continuous decline in catalase may be observed (fig. 6). When fruit is allowed to ripen on the tree both anabolic and katabolic processes are active, the relative importance of the two changing as the season advances. The products of katabolism in small amounts may then act as a stimulus to catalase activity, with the resultant U-shaped curve (fig. 4), which if prolonged until decomposition is well advanced would doubtless be S-shaped. Evidence that small amounts of decomposition products of ripening fruit may stimulate catalase activity is seen in figure 5. With mature fruit (fig. 5, *A*) visible ripening starts within a few days and catalase increases and then declines as decomposition products

accumulate. With immature fruit (fig. 5, *B*) visible ripening does not take place for several days and catalase decreases, and then increases as visible ripening begins with the resultant stimulatory action. If fruit is stored at 32°, decomposition products should be formed slowly, and catalase should increase slowly, as is shown in figure 7. That alcohol and ethylene chlorhydrin in small amounts may act as a stimulus to catalase activity has been shown (fig. 1). Table 2 shows that if the treatment is prolonged catalase activity will be lowered. Alcohol and acetaldehyde increase in fruit as it ripens, reaching a maximum in the overripe fruit. Fruit with a high acetaldehyde content has been found to be uniformly low in catalase.

Bunzell and Kenyon (*7*) have considered a possible reciprocal relationship between oxidase and catalase activity. They have advanced the theory that a portion of the colloidal constituents, such as proteins, may be responsible for the oxidase activity. A different type or degree of colloidality may be responsible for catalase activity, and the two types may be so interrelated that each tends to operate to the exclusion of the other. A reciprocal relationship doubtless sometimes occurs; however, if this relationship held universally, then the two enzymes would not parallel each other as Dexter (*12*) found with cabbage plants exposed to low temperature or as the present writers found with Bartlett pears during the growing season (p. 377). In certain cases conditions may be more favorable for the production of one type of enzyme than the other and a reciprocal relationship may appear to exist. If catalase and oxidase catalyze different reactions, then those conditions in which more of one enzyme would be needed may not necessarily be the same as for the other. Thus in spinach blight (*6*) additional oxidase may be produced by the plant, while with a different type of disease, such as soggy break-down of apple fruits (*19*), additional catalase may be produced. Until more is known about the part enzymes play in the cell and the reaction that each catalyzes, the reason for their increase will probably remain unknown.

In connection with the respiration-oxidase-catalase relationship, the work of Balls and Hale (*4*) is of interest. In 1932 they reported the discovery of an inactive form of catalase that could be activated by the addition of S–S derivatives (cystine, insulin, and oxidized glutathione). The S–S-containing compounds may be replaced by heart-muscle extract as reported by Swenson.[4] Swenson interpreted the presence of the inactive form as explaining the contradictory results of previous workers who attempted to correlate catalase activity with metabolic processes but failed to consider the temporarily inactive form.

With the addition of activators, Swenson found that the previously inactive form was like the original active form in being able to liberate oxygen from hydrogen peroxide but apparently differed from it in other respects. Thus, he found that there were either two forms of inactive catalase or, less probably, that two activators were necessary for its activation. Also, the inactive part is destroyed when the cell is killed by acetone, even though the activation takes place before the death of the cell. The original active form remains active after the cell is killed, but the recently activated form disappears. Working with bacteria, Swenson concluded that the difference was probably due to

[4] SWENSON, T. L. See footnote 3.

the manner in which the enzyme was attached to the cell. He found that the catalase content of bacteria varied with the oxygen supply and probably depended on the rate of cell oxidation. Most of the increased activity was of the inactive type. The increased catalase with the higher rate of oxidation lends support to the catalase-respiration relationship.

In interpreting the work of Swenson in relation to the hypothesis presented in this paper, it might be better to visualize the inactive catalase of Balls and Hale as a "proenzyme" (*42, p. 198*), i. e., catalase in various stages of development. At some stage in its formation, if an activator is added, it is able to decompose hydrogen peroxide in oxygen and water, although it is not fully stable catalase that can be extracted from the cell and treated as normally active catalase. This conception would be analogous, as mentioned above, to reserve food stored in a cell or plant. There is enough available food present normally to supply the immediate needs of the plant, but if an extra drain is made on it, as in germinating seeds, the reserve food in various forms is rapidly changed to a form that may be used in respiration; likewise, inactive catalase may exist as a proenzyme, and its availability for use may depend upon the needs of the plant or the presence of certain activators. Where the cell, as in bacteria, is exposed directly to the stimulant, oxygen, its response is very marked as evidenced by the rapid increase of the inactive catalase. The active catalase increased less rapidly, but probably fast enough to take care of any needs that were likely to develop.

Assuming that the reaction between activators and inactive catalase is a chemical one—and this conforms to modern enzyme theory—the efficiency of different activators would depend on the ability of the activator to join with the inactive catalase molecule and produce the active form. Thus Kolmer antigen would be able to supply more of the active catalase molecule in *Bacillus vulgaris* (Hauser) Migula (*Proteus vulgaris* Hauser) than would heart extract, i. e., it would activate more of the potential catalase in its various stages of formation and produce greater activity. If this explanation of activators is the correct one, it seems unlikely that there is any close correlation between catalase activity, including the inactive portion, and respiratory intensity, as proposed by Swenson. The measurement of the inactive form would depend upon the amount activated from the various stages of formation, and has been shown [5] to vary from one activator to another (Kolmer's antigen was more effective than heart activator); from one treatment to another (at 25° and 37° C. activation took place in 10 minutes' exposure, but at 0° it did not take place in 60 minutes' exposure); and from one organism to another (cystine for liver, heart extract for bacteria). Consequently, until the total potential catalase can be activated under various conditions and with various activators, it will be difficult to correlate respiratory intensity with the summation of the active and inactive forms. Otherwise the discovery of a more efficient activator or a more nearly perfect set of conditions for activation might completely invalidate the data secured. Since there is no evidence to support the theory that "inactive catalase" can function in the cell as active catalase or is available for use until changed to the active form, it seemed preferable to confine the work largely to the active form of catalase.

[5] SWENSON, T. L. See footnote 3.

The recent work of Stern (*39*) is of major interest to students of enzymology because of the direct evidence that he has obtained of the combination of the enzyme with the substrate. When monoethyl hydrogen peroxide is added to liver catalase the enzyme spectrum disappears and two new absorption bands appear. In a few minutes the bands disappear and the original enzyme spectrum reappears. The reappearance of the enzyme spectrum coincides with the disappearance of the titratable peroxide oxygen. No gas is evolved in the reaction. So far as the writers are aware, this is the first direct evidence not only of a combination of enzyme and substrate, but also of a combination between catalase and monoethyl hydrogen peroxide with the liberation of nascent oxygen. Molecular oxygen from hydrogen peroxide is usually assumed to be the normal catalase reaction. No change in the enzyme spectrum was observed when hydrogen peroxide was used as a substrate, the reaction probably being too rapid to be detected. If future work proves that catalase may liberate nascent oxygen in the living plant the present conception of plant respiration and catalase activity may have to be altered substantially.

SUMMARY

The oxidase and catalase activity of apple and pear fruits under various conditions has been studied in relation to the rate of respiration and to each other.

Oxidase and catalase activity were not directly correlated with rate of respiration or with each other, in fruit subjected to various storage temperatures or to various chemical respiratory stimulants or depressants.

There was a positive correlation between rate of respiration and oxidase and catalase activity in Bartlett pear fruits on the tree from the time they were very small until they reached commercial harvest maturity. When the fruits were permitted to remain on the tree past the normal season, the oxidase activity continued to decline while the rate of respiration and catalase activity increased.

There was a positive correlation between catalase activity and rate of respiration in Bartlett pear fruits as long as they remained on the tree.

The intercorrelation of respiration, catalase, and oxidase failed to hold after the fruit was removed from the tree and placed in storage. This was true whether the fruit was harvested relatively immature or whether it was allowed to reach full harvest maturity.

Chemical respiratory stimulants were not so effective with apple and pear fruits as has been reported with potatoes.

The interrelationship of oxidase, catalase, and rate of respiration is discussed. A possible explanation is suggested for the apparently conflicting results obtained by workers in this field.

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THE INFLUENCE OF VARIOUS FACTORS ON EGG PRODUCTION IN TURKEYS [1]

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INTRODUCTION

It has long been recognized that hereditary as well as environmental factors influence egg production in the domestic fowl. Following the work of Pearl (*9*),[3] Goodale (*2*) and later Hays (*3*) established the existence of several inherited factors, each influencing egg production. These factors include sexual maturity, winter pause, broodiness, persistency, and rate of production. Sexual maturity in chickens has usually been measured by age at first egg; winter pause by a period of nonproduction of 7 (or 4 days, Hays (*5*)) days or longer; broodiness by actual cessation of egg production due to the hen's becoming broody; persistency by the length of the laying year; and rate of production by a variety of measures such as percent production for the year or for a given period. These factors have been regarded as independent of each other except sexual maturity and persistency, which Hays (*4*) considers to be linked. Lerner and Taylor (*6*) have shown, however, that persistency as defined by Hays is determined partly by age at first egg, in which case the relation claimed by him would not hold. They used age at last egg as the measure of persistency, and found that, when the term is so defined, there is no evidence of linkage between persistency and sexual maturity. The object of the present paper is to determine how certain factors known to influence egg production in chickens may affect egg production in turkeys.

MATERIALS AND METHODS

The first-year records obtained by trap nesting all the turkeys in the breeding flock during a 3-year period were used. The total number of these records was as follows: For 1934, 91; for 1935, 74; and for 1936, 73. Of these, all the 1934 records were for birds of the Bronze variety; but the 1935 and 1936 records include a few for Bourbon Reds, Narragansetts, and cross-breds. The birds were hatched in April and May. None of them laid before January 1 of the following year. All had completed their first-year record, and all were apparently normal. Selection was not practiced in any way except to exclude a few birds that became sick or died.

The various measures of the factors to be considered were calculated from the trap nest and other records. These include date of hatch, calculated as the number of days from the first of the year in

[1] Received for publication September 23, 1937; issued April 1938.

[2] Dr. I. M. Lerner and Dr. L. W. Taylor kindly permitted the writer to refer to an unpublished manuscript of theirs. Dr. Lerner also rendered valuable assistance in connection with tables 2 and 3 which were partly calculated under the auspices of the Works Progress Administration.

[3] Reference is made by number (italic) to Literature Cited, p. 392.

which the bird was hatched; age at first egg, in days; date of first egg calculated as the number of days from the first of the year in which the record was made (in all cases the year following the one in which the bird was hatched); age at last egg, in days; date at last egg, in days from the first of the year in which the record was made; length of laying year, in days (= date of last egg minus date of first egg plus 1); length of all pauses [4] of 7 or more days; number of all pauses of 7 or more days; net annual rate measured by the number of eggs laid divided by the length of laying year less the number of days in all pauses of 7 days or more; net spring rate similarly calculated on the basis of eggs laid in March and April. The choice of a 7-day period of nonproduction as the minimum period recognized as a pause, was purely arbitrary. Marsden (*8*) has also, apparently arbitrarily, selected 5 days as the minimum period. No attempt was made to determine the best methods of calculating rate or the best measure of rate. March and April were, however, chosen for the spring period because rate of production, as calculated, was higher in these than in other months. Table 1 presents the means and standard deviations for first year egg production and for each of the factors correlated with first year egg production.

Coefficients of correlation were calculated by the methods described by Ezekiel (*1*) except for coefficients of multiple correlation, which were calculated by the method of Wallace and Snedecor (*11*).

TABLE 1.—*Mean and standard deviation for first-year egg production and the various factors correlated therewith*

Factor	1934		1935		1936	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
First-year egg production.....number..	77.2	30.9	76.1	30.3	79.4	29.8
Date hatched [1].....days..	124.9	14.2	111.7	14.7	115.4	14.1
Date of first egg [1].....do....	57.8	14.5	55.2	17.3	53.7	16.0
Age at first egg.....do....	296.9	18.8	308.3	20.7	303.0	29.3
Date of last egg [1].....do....	223.2	49.8	215.4	39.8	215.7	42.6
Age at last egg.....do....	464.7	54.4	471.1	46.2	464.9	42.7
Length of laying year.....do....	161.6	55.7	163.1	48.0	161.3	45.3
Total length of pauses.....do....	41.3	35.0	39.6	27.7	37.6	32.1
Pauses.....number..	4.4	1.7	2.3	1.6	2.1	1.4
Annual net rate.....percent..	61.4	6.8	61.3	8.5	64.6	7.3
Spring net rate.....do....	71.1	5.8	68.8	9.6	69.1	9.6

[1] Date is expressed in each case as the number of days from December 31. Thus the average date hatched was May 5 (124.9 days), April 22 (111.7 days), and April 25 (115.4 days).

STATISTICAL ANALYSIS

The zero order coefficients of correlation (r) are presented in table 2 together with $\bar{r}$, the coefficient most probably representing the true value for the universe from which the sample was drawn. (See Ezekiel (*1*).) The objects of presenting the data in this table are: (1) To determine which of the factors considered influence annual egg production; (2) to determine which measure should be used when more than one measure of a factor is available (for example, date of first egg and age at first egg as measures of sexual maturity); (3) to

[4] The word "pause", as used herein, includes broody and nonbroody periods. The majority of the birds that paused did not go broody.

determine whether the factors correlated with egg production are correlated with, or are independent of, each other.

Date of hatch has, apparently, no consistent influence on egg production. This conclusion would probably not apply to birds hatched late in the season. Of the two measures of sexual maturity, date of first egg is more closely correlated with egg production than is age at first egg. Date of first egg would seem, therefore, to be the more suitable measure of sexual maturity. Date of last egg and age at last egg are both highly and about equally correlated with egg production. Date of last egg has therefore been selected as the measure of persistency to be uniform with the measure of sexual maturity (date of first egg).

TABLE 2.—*Zero order coefficients of correlation*

Variables correlated	1934		1935		1936	
	r	$\bar{r}$	r	$\bar{r}$	r	$\bar{r}$
First-year production; date hatched	−0.229	−0.205	−0.339	−0.320	0.205	+0.169
First-year production; date of first egg	−.460	−.450	−.518	−.508	−.311	−.290
First-year production; age at first egg	−.170	−.134	−.193	−.154	−.367	−.350
First-year production; date of last egg	.654	.649	.648	.642	.572	.564
First-year production; age at last egg	.679	.675	.667	.661	.520	.510
First-year production; length of laying year	.715	.711	.726	.721	.681	.675
First-year production; length of pauses	−.212	−.186	−.327	−.307	−.395	−.380
First-year production; number of pauses	.059	0	−.071	0	−.060	0
First-year production; annual rate	−.104	0	.273	.248	.176	.132
First-year production; spring rate	.263	.242	.401	.386	.337	.318
Length of pauses; date of first egg	−.025	0	.063	0	.125	.042
Length of pauses; date of last egg	.475	.466	.357	.340	.410	.395
Length of pauses; length of laying year	.452	.442	.259	.233	.320	.300
Length of pauses; spring rate	.105	0	.187	−.147	−.011	0
Spring rate; date of first egg	−.012	0	.117	0	.100	0
Spring rate; date of last egg	.168	.132	.159	.108	.233	.202
Spring rate; length of laying year	.138	.028	.028	0	.155	.101
Spring rate; annual rate	.587	.581	.773	.756	.679	.673
Length of laying year; date of first egg	−.381	−.368	−.659	−.653	−.262	−.236
Length of laying year; date of last egg	.963	.963	.888	.886	.913	.912
Date of first egg; date of last egg	−.127	−.071	−.226	−.195	.088	0

Length of laying year is more closely correlated with egg production than date of last egg. As pointed out by Lerner and Taylor (*6*), length of laying year depends upon age at (or date of) first and last egg, which in the present work, in complete agreement with their data for chickens, are independent variables (table 2). That date of first egg and date of last egg determine the length of the laying year is shown by the coefficients of multiple correlation for these three variables, which ranged from 0.976 to 1.000. Length of laying year, therefore, cannot logically be used as a measure of persistency.

Length of pauses is correlated with egg production, whereas number of pauses is not. Length of pauses is influenced by date of last egg. There is also a significant correlation between length of pause and length of laying year, but this obviously results from the close association between the latter and the date of last egg.

Net rate for the vear is not correlated with egg production, whereas there is a significant correlation between egg production and net spring rate. Spring rate and annual rate, though correlated, appear to be independent of other variables, except, perhaps, date of last egg.

The data in table 1 show that sexual maturity (date of first egg), persistency (date of last egg), pauses (length of pauses), and rate (net spring rate) influence egg production. As measured by the coefficient of simple correlation, date of last egg is the most important of the factors considered. The coefficients of multiple correlation (R) are presented in table 3, $\overline{R}$ giving the most probable value for the universe from which the sample was drawn. The coefficient of multiple correlation for date of first egg and date of last egg with egg production has about the same magnitude as the coefficient of simple correlation for length of laying year and egg production. Likewise, when length of laying year is used instead of date of first egg and date of last egg with other variables, the coefficients of multiple correlation are about the same as when the date of first egg and date of last egg are used. Evidently, then, as already pointed out, the influence of length of laying year on the egg production of turkeys is simply the combined influence of date of first egg and date of last egg, the two independent variables that determine the length of the laying year.

TABLE 3.—*Coefficients of multiple correlation*

Egg production correlated with—	1934		1935		1936	
	R	$\overline{R}$	R	$\overline{R}$	R	$\overline{R}$
Date of first egg; date of last egg	0.756	0.750	0.752	0.744	0.683	0.671
Date of first egg; length of pauses	.511	.494	.595	.580	.475	.451
Date of first egg; spring rate	.528	.512	.696	.686	.486	.462
Date of last egg; length of pauses	.884	.881	.882	.879	.897	.894
Date of last egg; spring rate	.672	.663	.716	.706	.610	.595
Length of pauses; spring rate	.356	.327	.477	.454	.518	.497
Date of first egg; date of last egg; length of pauses	.953	.951	.928	.925	.918	.914
Date of first egg; date of last egg; spring rate	.773	.764	.837	.829	.720	.705
Date of first egg; length of pauses; spring rate	.585	.574	.726	.712	.598	.575
Date of last egg; length of pauses; spring rate	.900	.897	.894	.889	.907	.903
Date of first egg; date of last egg; length of pauses; spring rate	.970	.969	.953	.950	.959	.957
Length of laying year; length of pauses	.934	.932	.900	.807	.896	.893
Length of laying year; spring rate	.733	.725	.820	.814	.732	.723
Length of laying year; length of pauses, spring rate	.954	.952	.943	.940	.970	.969

In general, when egg production is correlated with two variables, the coefficient of multiple correlation so obtained is higher than the coefficient of simple correlation for egg production and either one of these variables (tables 2 and 3). When egg production is correlated with three variables, the coefficients of multiple correlation are still higher; and when egg production is correlated with all four variables, the highest coefficient of multiple correlation is obtained. The magnitude of the coefficient of multiple correlation obtained when egg production is correlated with all four variables is so close to 1 as to indicate that they account for substantially all the variance in first-year egg production in these groups of turkeys.

The relative importance of the four variables that have been shown to influence egg production can, as pointed out by Lerner and Taylor (7), be determined in several ways. The one adopted here is the coefficient of partial determination that measures the reduction in variance, due to the added factor, after the effects of the other independent factors have been accounted for (table 4).

TABLE 4.—*Reduction in unexplained variance, for factors considered, 1934–36*

Egg production correlated with—	Factors already considered	1934	1935	1936
Date of first egg	Date of last egg; length of pauses	0.571	0.366	0.180
Date of last egg	Date of first egg; length of pauses	.873	.783	.793
Length of pauses	Date of first egg; date of last egg	.781	.677	.701
Date of first egg	Date of last egg; spring rate	.257	.376	.221
Date of last egg	Date of first egg; spring rate	.436	.408	.361
Spring rate	Date of first egg; date of last egg	.048	.298	.085
Date of last egg	Length of pauses; spring rate	.782	.736	.755
Length of pauses	Date of last egg; spring rate	.652	.582	.714
Spring rate	Date of last egg; length of pauses	.119	.075	.081
Date of first egg	Length of pauses; spring rate	.249	.379	.111
Length of pauses	Date of first egg; spring rate	.091	.068	.160
Spring rate	Date of first egg; length of pauses	.112	.258	.149
Date of first egg	Date of last egg; length of pauses; spring rate	.670	.553	.544
Date of last egg	Date of first egg; length of pauses; spring rate	.909	.801	.874
Length of pauses	Date of first egg; date of last egg; spring rate	.853	.816	.833
Spring rate	Date of first egg; date of last egg; length of pauses	.365	.319	.489

The values obtained in the different years differ somewhat just as did those presented in tables 2 and 3. The order of the magnitudes is in good agreement for all 3 years. The data presented indicate that the relative importance of the influence of the four variables considered in determining first-year egg production is in the following order: Date of last egg, length of pauses, date of first egg, and spring rate. Date of last egg appears to be definitely the most important single factor determining egg production, whereas rate appears to be of least relative importance. Net spring rate does, however, exert a significant, if small, influence on egg production. Although length of pause is correlated with date of last egg, each of these factors exerts an influence on egg production apart from that of the others. (See tables 3 and 4.)

DISCUSSION

The data presented in this paper agree with those available for chickens. This agreement points to the conclusion that the internal factors influencing egg production are similar. Egg production in turkeys may therefore be regarded as a complex of characters that are probably influenced by a number of genes just as in the domestic fowl.

As has been pointed out above, the time factors (date of first egg, pauses, and date of last egg) have a greater influence on egg production than the rate factor. One might infer that the factors governing egg production in turkeys differ from those governing egg production in chickens, except that Lerner and Taylor (*7*) have obtained similar results with nonpausing populations of Single-Comb White Leghorns. Though there may prove to be some significant differences in sexual maturity in turkeys and chickens, the data presented in this paper as well as those of Marsden (*8*) and others indicate that the resemblances are much greater than the differences.

The difference in the best measure of sexual maturity (age at first egg in chickens, date of first egg in turkeys) is significant. It points to a greater influence of environment on egg production among turkeys than among chickens. The case has been stated in a different way by Scott and Payne (*10*), who point out that the reproductive cycle of turkeys resembles more closely that of birds in the wild state than it does that of other domesticated farm birds. Scott and Payne

found that housing, and factors in the environment other than light, had no influence on the egg production of turkeys. The implied difference in the sexual maturity of chickens and turkeys, therefore, probably does not result from the fact that the turkeys were not housed and that chickens invariably are.

Though persistency (date or age at last egg) has the greatest influence on annual egg production in turkeys, other factors may be of equal importance because of their influence on egg production during the breeding season. Further data are needed to determine whether such is the case.

SUMMARY

A statistical analysis was made of the first-year records of 238 turkeys which were trap-nested in 1934, 1935, and 1936 and which had in each case been hatched in April and May of the preceding year.

Date of hatch was not found to have any significant, consistent influence on egg production.

Date of first egg was more closely correlated with egg production than was age at first egg. Date of first egg was accordingly selected as the more suitable measure of sexual maturity.

Date of (and age at) last egg was more closely correlated with egg production than was any other single factor. Date of first egg was not correlated with date of last egg.

Length of laying year was correlated with egg production, the zero order coefficients having about the same magnitude as the coefficients of multiple correlation for egg production and the two variables (date of first and date of last egg) that determine length of laying year. The coefficient of multiple correlation for length of laying year with date of first egg and date of last egg ranged from 0.976 to 1.000.

Total length (but not number) of pauses was correlated with egg production. Length of pause was correlated with date of last egg, but not with date of first egg nor with spring rate.

Net spring (March and April) rate was correlated with egg production, whereas annual net rate was not. Spring rate was not correlated with date of first egg, date of last egg, or length of pauses.

The coefficients of multiple correlation of egg production with date of first egg, date of last egg, length of pauses, and net spring rate approached the maximum possible value, varying from 0.950 to 0.970, a fact indicating that these four factors determine most, if not all, of the variance in first-year egg production.

The four factors found to influence annual egg production, particularly as measured by the coefficient of partial determination, are, in order of decreasing importance: (1) Date of last egg (persistency), (2) length of pause, (3) date of first egg, (4) net spring rate.

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A MORPHOLOGICAL STUDY OF FLOWER AND SEED DEVELOPMENT IN PEPPER[1]

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INTRODUCTION

Of the many economically important vegetable crop plants, few have been more neglected from the standpoint of type improvement than the pepper (*Capsicum frutescens* L.). Many of the common varieties are lacking in uniformity of fruit shape, in thickness of flesh, in smallness of placenta, and in color. Most varieties are also susceptible to certain destructive diseases, some of which could be controlled by the use of scientific methods of breeding and selection in producing resistant strains. Before any improvement program is undertaken through breeding methods, however, the morphology of the flower should be better understood. A knowledge of the method and time of pollination, the time required for fertilization, and the behavior of the flower throughout its development are also important.

MATERIALS AND METHODS

The World Beater variety of pepper was used exclusively for this study. Some of the plants from which material was collected were grown in the experimental greenhouses of the Department of Vegetable Crops of the New York State College of Agriculture at Ithaca, N. Y., during the fall and winter of 1932–33; others were grown in the horticultural greenhouses of the Georgia Experiment Station at Experiment, Ga., during the winter of 1935–36. The material for study was killed and fixed in a solution made up of solution A, consisting of 4 parts of commercial 40 percent formalin and 1 part of water, and solution B, consisting of 1 g of chromic acid and 10 cc of acetic acid in 90 cc of water. Equal parts of these two solutions were mixed at the time they were used. The material was then dehydrated in alcohol and embedded in paraffin, xylol being used according to the usual laboratory method. Both transverse and longitudinal sections were cut, 10μ to 12μ in thickness, mounted serially, and stained in Heidenhains' iron-alum haematoxylin. All figures were drawn with the aid of a camera lucida.

DEVELOPMENT OF THE FLOWER

Small (*21, p. 992*)[3] describes the pepper flower as being solitary in the axils or in small cymes. The calyx is barely accrescent and has five wholly or partly united sepals. The corolla is usually white,

[1] Received for publication September 2, 1937; issued May 1938. Paper No. 49, Journal Series of the Georgia Agricultural Experiment Station.

[2] The author is indebted to Dr. Ora Smith of Cornell University for valuable advice and constructive criticisms during the entire course of the work; to Dr. F. F. Cowart for preparing some of the material for microscopic study; and to Drs. B. B. Higgins and W. A. Jenkins for their interest and assistance in the investigation.

[3] Reference is made by number (italic) to Literature Cited, p. 418.

nearly rotate, five-lobed, and imbricated. There are typically five stamens adnate to the base of the corolla. The anthers are bluish and dehisce by longitudinal splitting. The stigma is club-shaped or dilated.

The time of flower primordia differentiation in the pepper was found by Auchter and Harley (*1*) to be influenced to some extent by photoperiod. More recently Cochran (*7*) found that temperature, as well as photoperiod, was of great importance. The protuberance of the first or terminal flower of the cyme arises in the axil of a leaf and in some cases of a branch. The two succeeding flowers of the cyme each

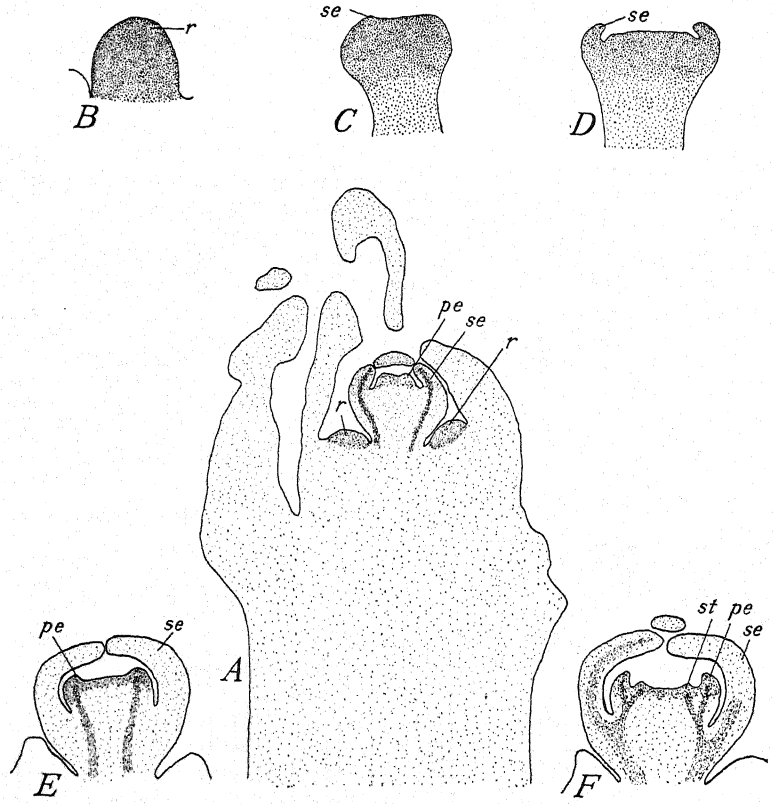


FIGURE 1.—*A*, Longitudinal section of developing flower of cyme. *B*, *C*, *D*, Meristematic region of receptacles: *B*, no floral organs differentiated; *C*, *D*, sepals differentiating. *E*, Early development of petals. *F*, Sepals, petals, and stamens developing. *r*, receptacle; *se*, sepals; *pe*, petals; *st*, stamens. All × 225.

arise from similar protuberances which have grown out from the base of the pedicel of the preceding flower (fig. 1, *A*). The growing apex or protuberance is at first somewhat conical (fig. 1, *B*), but subsequently becomes flattened and broadened (fig. 1, *C*). The primordia of the first floral organs arise on the periphery of the flattened meristematic protuberance, indicating the beginning of the sepal lobes (fig. 1, *C*, *D*). These rudimentary sepals curve inward, arching over the apex of the axis. Primordia of the second whorl soon appear in the same manner but are slightly higher on the axis and are alternate with the sepal lobes. These give rise to the petals (fig. 1, *E*). Subsequently

there appears on the axis and nearer the center, opposite the sepal lobes and alternate with the petals, a third set of small protuberances

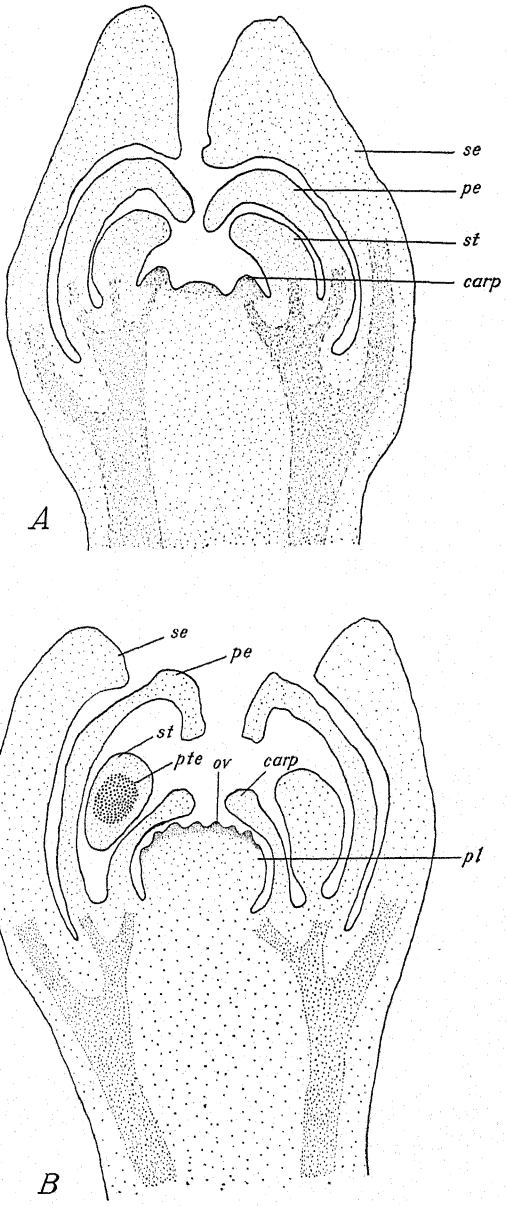


FIGURE 2.—*A*, Initiation of carpels and development of sepals, petals, and stamens, × 225; *B*, longitudinal section of young flower showing further development of sepals, petals, stamens, and carpels; young ovules in macrospore mother-cell stage and microspores in tetrad stage, × 440; *se*, sepals; *pe*, petals; *st*, stamens; *carp*, carpels; *pte*, pollen tetrads; *ov*, ovules; *pl*, placenta.

which develop into the stamens (fig. 1, *F*). The carpels are the last cycle of flower organs to differentiate. They arise from the center of

the floral axis and develop into a compound pistil consisting usually of three united carpels (fig. 2, *A*, *B*). A pepper flower in transverse section is shown in figure 4, *A*. The petals and stamens curve inward over the top of the carpels. These observations are in complete agreement with those of Béla (*2*) for the pepper and with Cooper (*10*) and Smith (*23*) for the tomato.

MACROSPOROGENESIS AND DEVELOPMENT OF THE MACROGAMETOPHYTE

The young ovules are first distinguishable as small, rapidly growing protuberances which arise from the placental tissue (fig. 2, *B*; fig. 3, *A*, *B*, *C*). Occasionally internal abnormalities, the description and morphology of which have been described by Cochran (*8*), arise simultaneously with the young ovules and for a while cannot be distinguished from the normal ovules. The ovules soon become somewhat pointed (fig. 5, *A*) as the result of more rapid cell division on one side of the ovule than on the other, but eventually they become anatropous in form. This type of ovule is the commonest among the modern flowering plants, and has been found by Chatin (*6*), Cooper (*11*), Bhaduri (*3*), and Smith (*23*) to be typical for various other members of the Solanaceae. When the developing ovule has attained the stage shown in figure 4, *C*, the hypodermal archesporial cell, which functions as the macrospore mother cell in pepper, has already become differentiated in the nucellus. This occurs a little before the initiation of the integument. The archesporial cell is distinguishable on account of its large size, its conspicuous nucleus, and heavily stained cytoplasm. The macrospore mother cell becomes several times larger than the hypodermal archesporial cell from which it arises and is enclosed by a single-layered nucellus on the sides and apex (fig. 5, *B*).

The primordium of the integument is not evident until after the differentiation of the archesporial cell. The integument arises as an outgrowth from the base of the nucellar tissue and continues to develop until it finally surrounds the nucellus and elongates so as to form a micropyle some 8 to 10 cells in length (fig. 5, *B*, *C*; fig. 6, *A*). Further growth of the nucellus occurs by anticlinal division of the cells. The nucellus consists of a single layer of cells and is thought to nourish the nascent embryo. It completely degenerates as the embryo sac develops.

The much enlarged macrospore mother cell, by two successive divisions, gives rise to a linear row of four macrospores of approximately the same size (fig. 5, *B*, *C*; fig. 6, *A*). The three macrospores nearest the micropyle soon degenerate, leaving the macrospore at the chalazal end to function as the one-celled embryo sac (fig. 6, *B*, *C*). Before the first division of its nucleus the functioning macrospore increases rapidly in size. Simultaneously with this rapid growth, however, the cells of the nucellus gradually become disorganized and finally disappear (fig. 7, *A*, *B*, *C*). The work of Young (*28*) shows that the nucellus cells in the potato fail to degenerate. In a more recent study of macrosporogenesis and development of the macrogametophyte in the potato, however, Rees-Leonard (*19*) has shown that cells of the nucellus disappear in practically the same manner as described in this study for pepper.

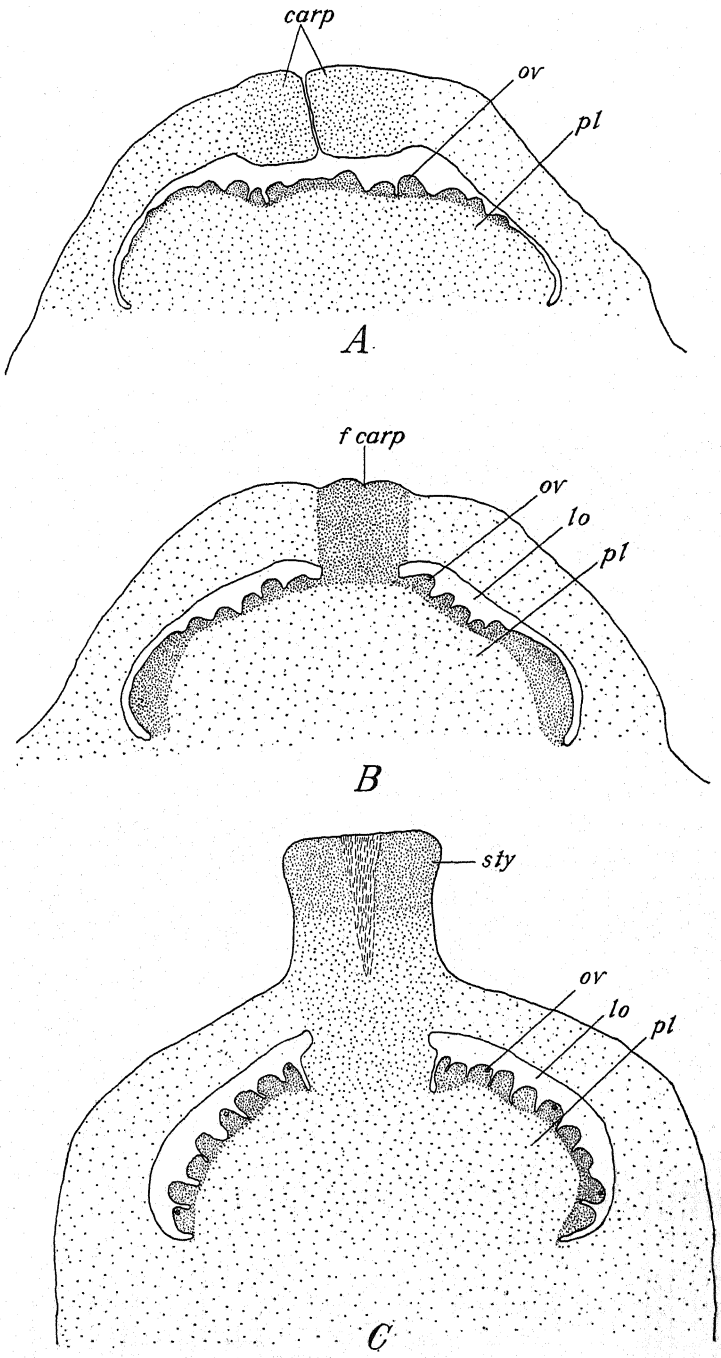


FIGURE 3.—*A*, Carpels just prior to fusing; developing ovules. *B*, Carpels fused, setting off two loculi; style development started. *C*, Style development complete; ovules further developed. *carp*, carpels; *ov*, ovules; *pl*, placenta; *f carp*, fused carpels; *lo*, loculus; *sty*, style. All × 440.

The first gametophytic division occurs in the central portion of the embryo sac (fig. 6, *C;* fig. 7, *A*). The resulting daughter nuclei separate, one migrating toward the mocropylar end and the other toward the chalazal end of the embryo sac (fig. 7, *B*). By two subsequent successive divisions, the two daughter nuclei produce eight

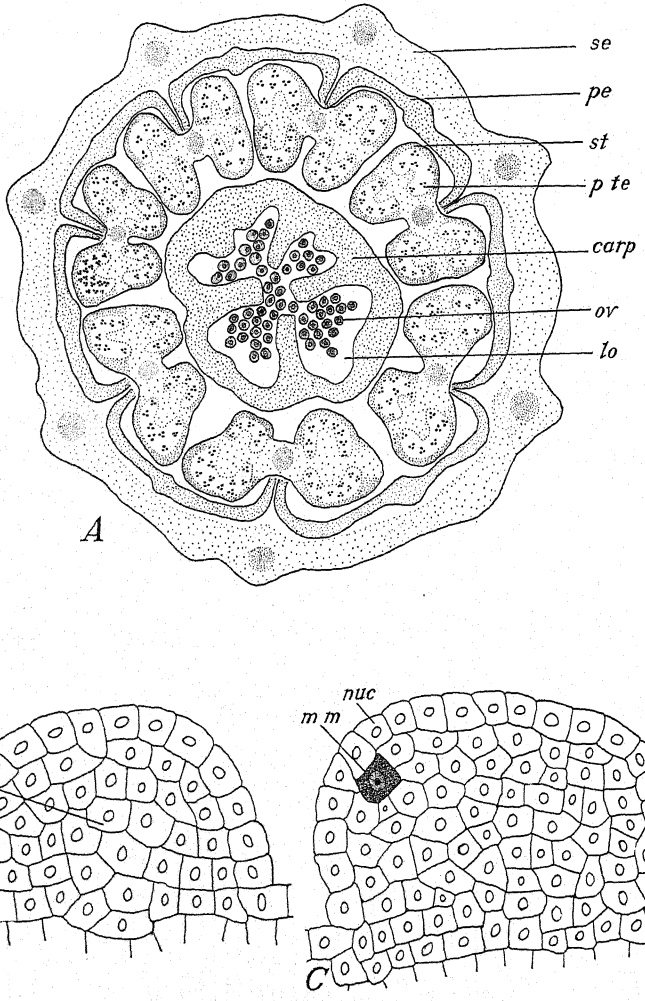


FIGURE 4.—*A*, Transverse section of pepper flower at the macrospore mother-cell and microspore tetrad stages; same stage as shown in figure 2, *B*. Note presence of seven stamens; five is the usual number. × 440. *B*, Young ovule soon after having arisen from placental tissue. × 950. *C*, Developing ovule with macrospore mother cell. × 950. *se*, Sepals; *pe*, petals; *st*, stamens; *p te* pollen tetrads; *carp*, carpels; *ov*, ovule; *lo*, loculus; *m m*, macrospore mother cell; *nuc*, nucellus.

nuclei. Two of the eight nuclei take a position near the center of the sac and become the polar nuclei; three migrate to the chalazal end of the sac and become the antipodals; and the three remaining nuclei take a position near the micropylar end of the sac. Of these the two more pear-shaped ones become the synergids, and the rather spherical one the egg (fig. 7, *D*). Guignard (*15*) as early as 1882 studied the development of the female gametophyte in some solanaceous plants

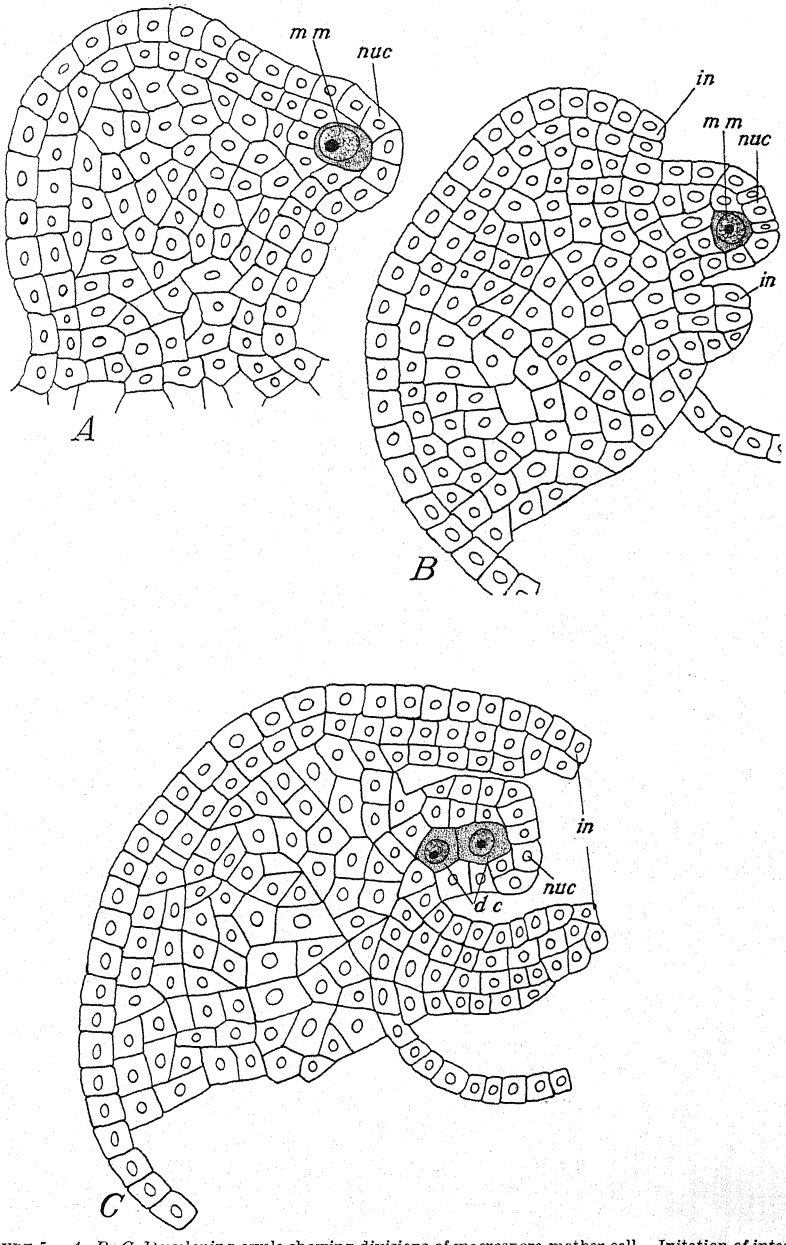


FIGURE 5.—*A*, *B*, *C*, Developing ovule showing divisions of macrospore mother cell. Initation of integument shown in *B*, *C* shows further growth of integument and two daughter nuclei as a result of the first division of the macrospore mother cell. *m m*, Macrospore mother cell; *nuc*, nucellus; *in*, integument; *d c*, daughter cells. All × 950.

among which were included *Nicotiana tabacum* and *Cestrum splendens*. He concluded that the development of the embryo sac in these plants follows the normal type or the same as that reported here. Banerji,[4] working with *Capsicum annuum*, and Cooper (*11*) and Smith (*23*),

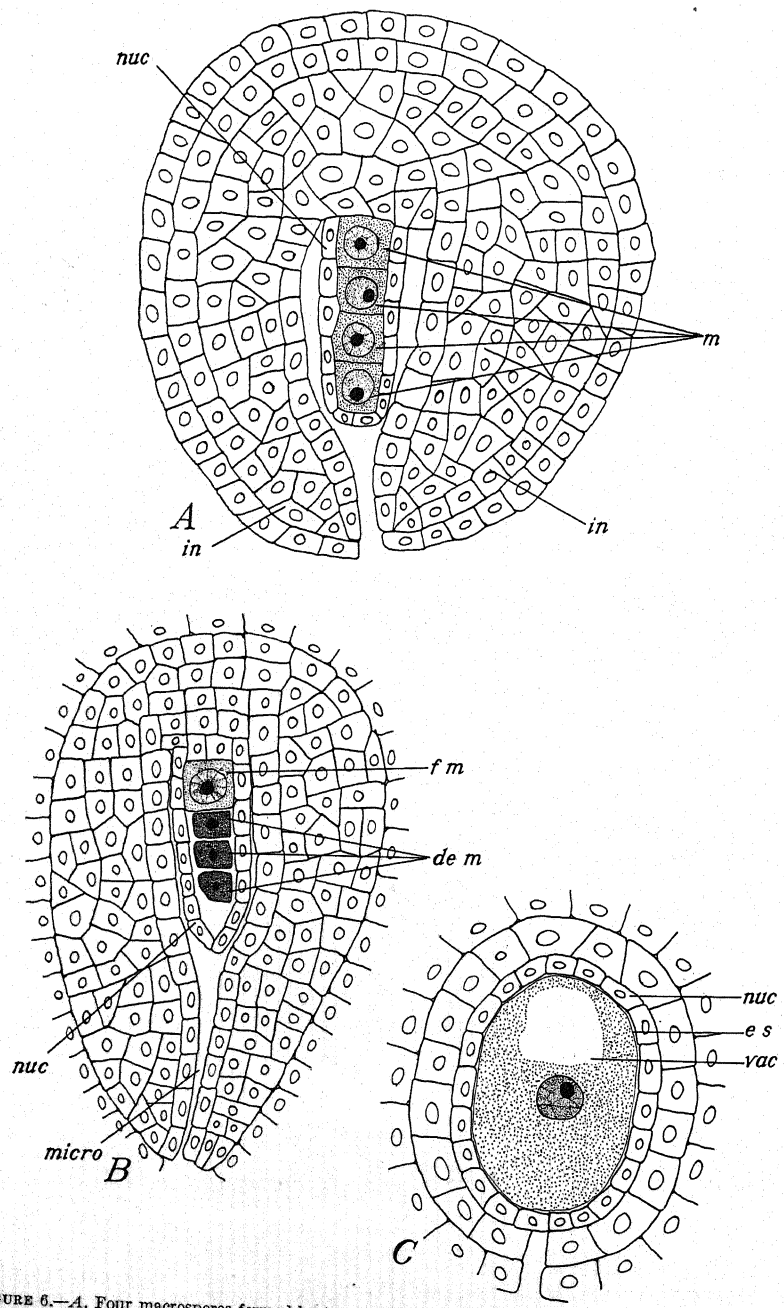


FIGURE 6.—*A*, Four macrospores formed by two successive divisions of the macrospore mother cell. *B*, Tetrad of macrospores, showing the functioning and three degenerating macrospores. *C*, one-nucleate embryo sac. *nuc*, Nucellus; *m* macrospores; *in*, integument; *f m*, functioning megospore; *de m*, degenerating macrospore; *e s*, embryo sac; *vac*, vacuole; *micro*, micropyle. All × 950.

with *Lycopersicon esculentum*, found the same type of development of the embryo sac. Soon after the development of the mature or eight-nucleate embryo sac, the antipodal cells begin to degenerate,

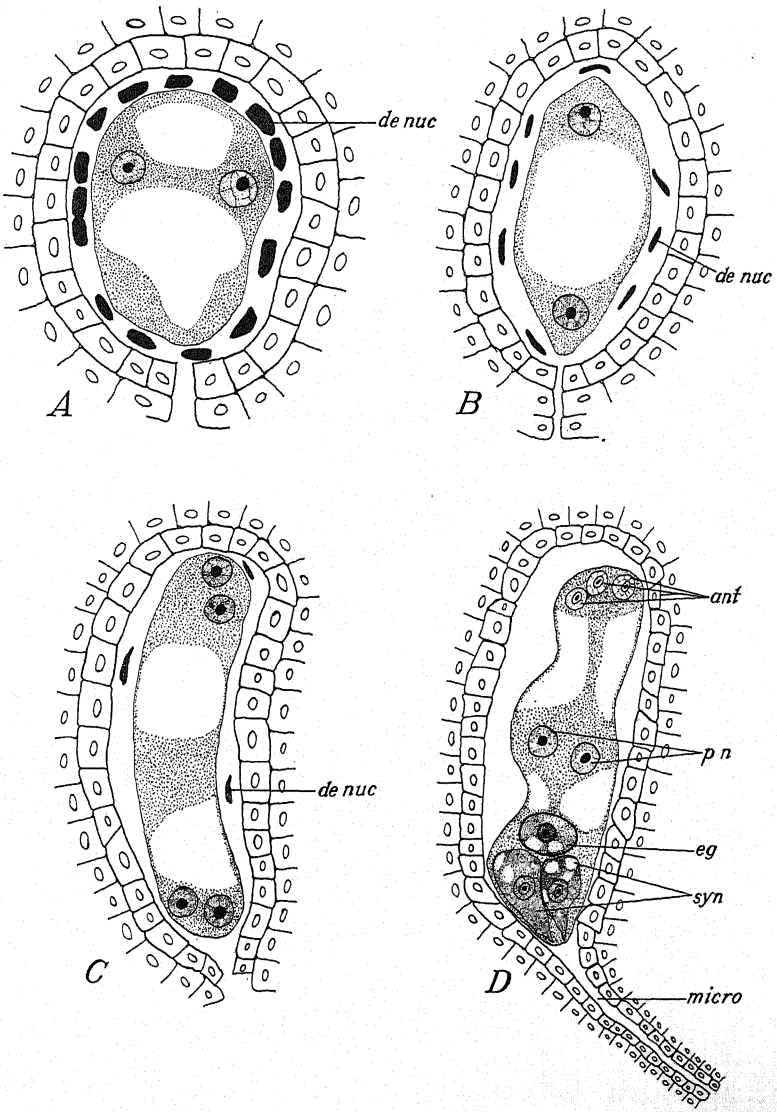


FIGURE 7.—*A*, *B*, Two-nucleate embryo sac; nucellus beginning to degenerate. *C*, Four-nucleate embryo sac; nucellus practically gone. *D*, Eight-nucleate or mature embryo sac. *de nuc*, Degenerating nucellus; *ant*, antipodal nuclei; *p n*, polar nuclei; *eg*, egg; *syn*, synergids; *micro*, micropyle. All × 640.

and by the time fertilization has taken place, only distorted portions of them remain. Fusion of the polar nuclei takes place just before or at the time of fertilization.

MICROSPOROGENESIS AND DEVELOPMENT OF THE MICROGAMETOPHYTE

Prior to any archesporial differentiation, the anthers consist of a homogeneous mass of meristematic tissue (fig. 8, *B*). However, when the young stamens have reached the stage of development shown in figure 2, *A*, a single row of hypodermal archesporial cells can be seen. These cells are easily distinguished by their deeply stained contents and large nuclei. The archesporial cells (fig. 8, *C*) first divide periclinally (fig. 8, *D*), thus giving rise to a single row of pollen mother cells on the inside and an outer layer of similar cells which divide to form the tapetum, the middle layer, and the endothecium (fig. 8, *E*). The tapetal cells are at first uninucleate, but subsequently divide and become binucleate (fig. 9, *A*). Soon after the sporogenous cells begin to separate and round off, the cells of the tapetum begin to enlarge and change their original shape (fig. 9, *B*, *C*). The cells are not long; some enlarge tangentially as well as radially and appear somewhat square, while others are irregular in shape. At the time of the late anaphase of the first meiotic division of the pollen mother cells, the tapetal cells show signs of degeneration. As they grow older they gradually lose their contents which are supposedly absorbed by the developing pollen. They are quite distorted by the time the pollen tetrad stage is reached and subsequently collapse entirely.

The first signs of degeneration of the middle layer are noted during the metaphase of the first meiotic division. These cells seem to degenerate in the beginning at a faster rate than do the tapetal cells, although they are not entirely absorbed even at the time of pollen tetrad formation. Cells of the endothecium do not undergo the above-described change.

In figure 9, *D*, is shown the pollen mother cells in the telophase of the second meiotic division with cell walls just beginning to form. At this time the macrogametophyte has started to enlarge, and is in the macrospore mother-cell stage of development. Tetrads rounded off and enclosed within the pollen mother cell wall are to be seen in figure 10, *A*. At the time they are set free from the enclosure, the walls of the microspores are very thin, but later they become considerably thickened (fig. 10, *B*, *C*). The microspore nucleus, which contains the reduced number of chromosomes, divides to two, around one of which a membrane cuts out a generative cell. The other nucleus develops into the vegetative or tube cell, which later grows out to form the pollen tube. The generative cell divides to form two male gametes within the growing pollen tube before the latter reaches the embryo sac. Mature microgametophytes are to be seen in figure 10, *C*. Many microspores develop within the loculi of each anther.

ANTHESIS

A close study of anthesis and fruit setting in *Capsicum frutescens* was made by the writer under both greenhouse and field conditions at Ithaca, N. Y., over a 4-year period. Factors considered in this work included temperature, soil moisture, soil nitrogen content, atmospheric humidity, and photoperiod. The first- and last-named factors proved to have the greatest influence on anthesis.

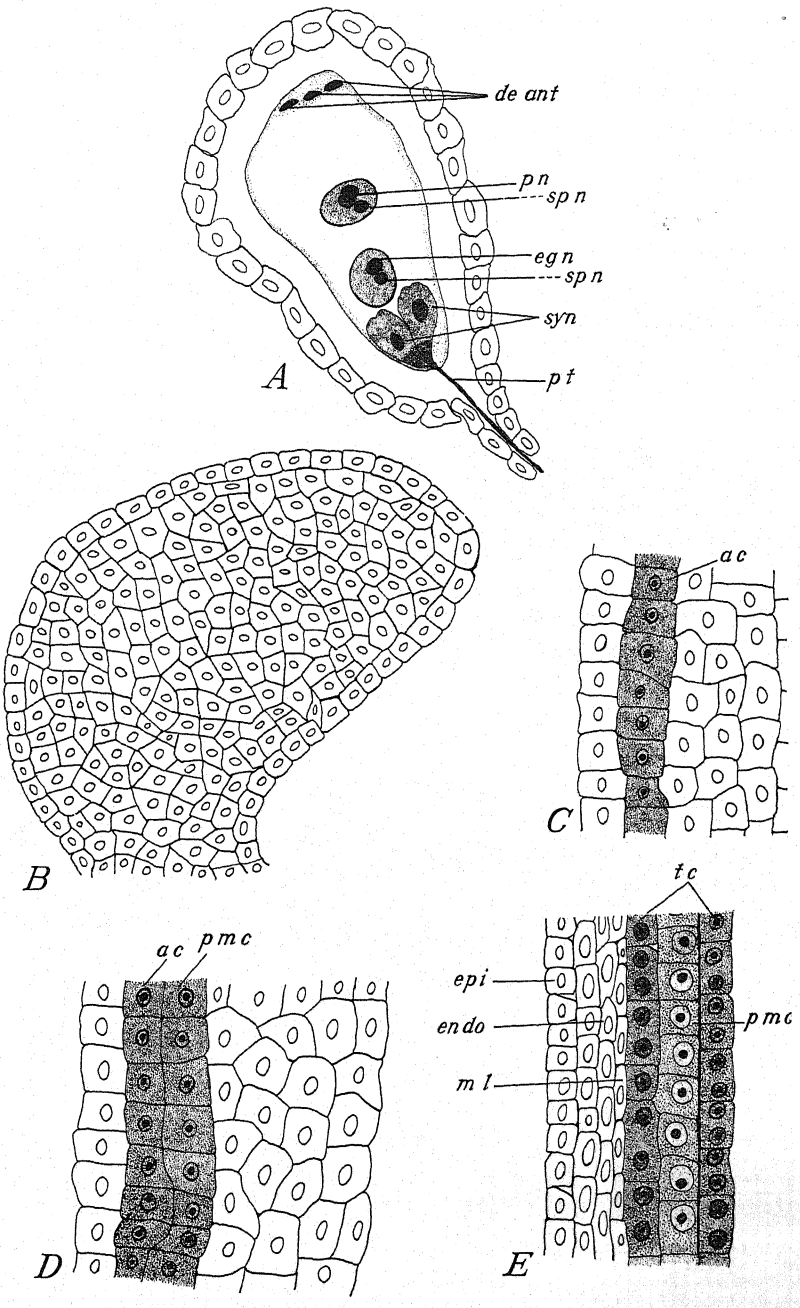


FIGURE 8.—*A*, Fertilization 42 hours after pollination; antipodals degenerating; triple fusion of two polar nuclei with sperm nucleus; egg nucleus fused with other sperm nucleus. × 950. *B*, Longitudinal section of young anther prior to differentiation of archesporial cells. × 225. *C*, Row of archesporial cells in anther. × 640. *D*, Archesporial cells divided, cutting off row of pollen mother cells on inside. × 640. *E*, Row of pollen mother cells enclosed by two rows of two-nucleate tapetal cells. × 640. *de ant*, Degenerating antipodals; *p n*, polar nuclei; *sp n*, sperm nucleus; *eg n*, egg nucleus; *syn*, synergids; *p t*, pollen tube; *a c*, archesporial cells; *p m c*, pollen mother cells; *epi*, epidermis; *endo*, endothecium; *m l*, middle layer; *t c*, tapetal cells.

Plants in the greenhouse were grown from seed to maturity under four different temperatures, namely, 50° to 60°, 60° to 70°, 70° to 80°, and 90° to 100° F. All other conditions were kept as nearly optimum for growth as possible. Long and normal day conditions were main-

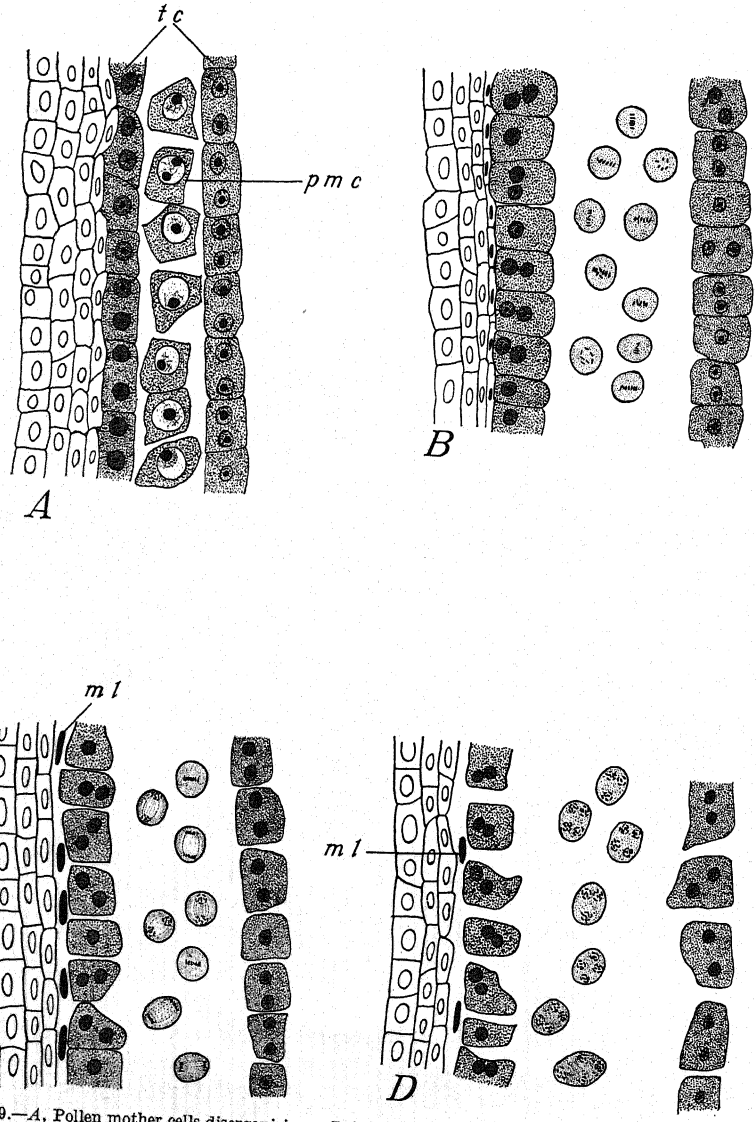


FIGURE 9.—*A*, Pollen mother cells disorganizing. *B*, Metaphase of first meiotic division of pollen mother cells. *C*, Late anaphase of first meiotic division of pollen mother cells; tapetal cells and middle layer degenerating. *D*, Late telophase of second meiotic division of daughter cells. *t c*, Tapetal cells; *p m c*, pollen mother cells; *m l*, middle layer. All × 950.

tained under each of these temperatures. The results of these studies, which have been published elsewhere (*9*), show that anthesis occurred sooner at the higher temperatures to which the plants were exposed. At 50° to 60° the time that elapsed between seeding and anthesis

was 135 days, whereas at 90° to 100° it was only 55 days. Anthesis resulted in 84 days at 60° to 70° and in 73 days at 70° to 80°. Similar

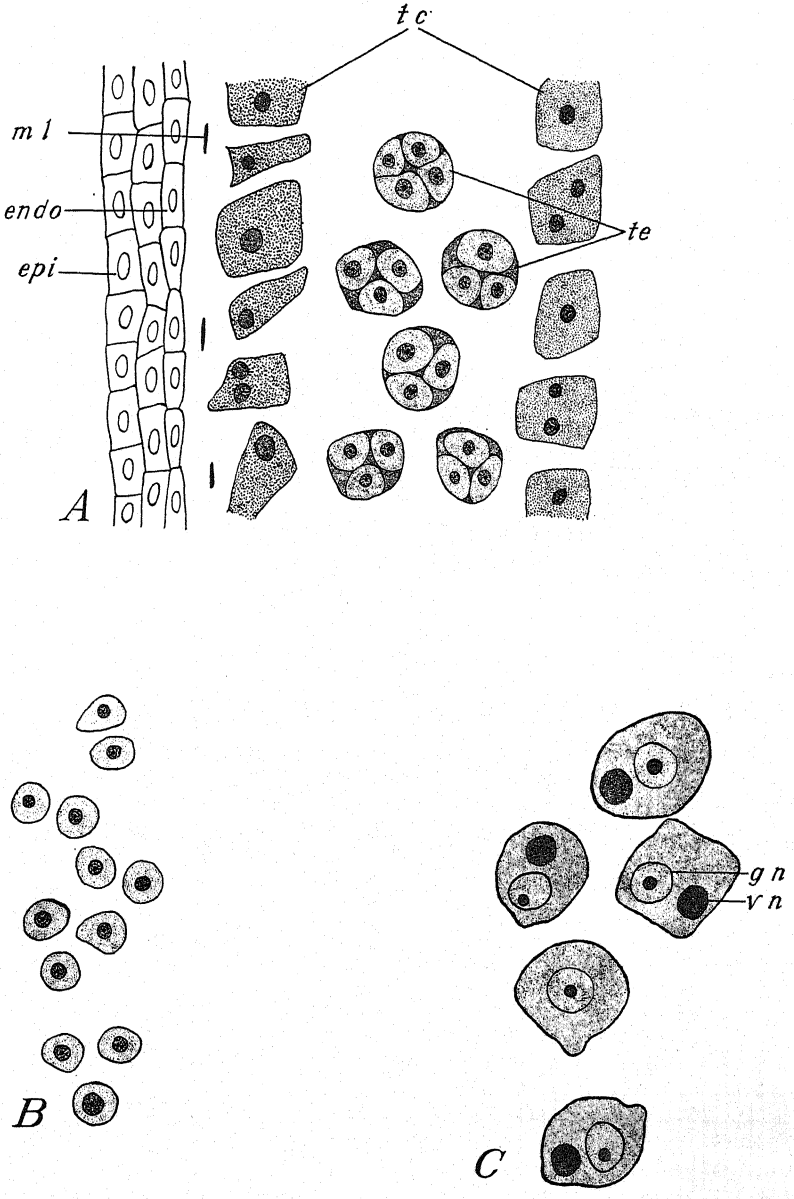


FIGURE 10.—*A*, Tetrads enclosed within the walls of the pollen mother cell. × 950. *B*, Young pollen grains just after being freed from tetrad encasement. × 640. *C*, Mature pollen grains showing one generative and one vegetative nucleus. × 950. *m l*, Middle layer; *endo*, endothecium; *epi*, epidermis; *tc*, tapetal cells; *te*, tetrad; *g n*, generative nucleus; *v n*, vegetative nucleus.

results with tomato have been reported by Smith (*22*). Anthesis occurred 10 days earlier under a 10-hour day than under a 14-hour

day. Pepper is therefore a "short-day" type of plant, as the term is used for plants that produce their flower primordia in response to short-day conditions.

Erwin (*14*) made an interesting and thorough study to determine at what time during the day anthesis occurs in the pepper, and he concluded that the major portion of the blossoms open within 3 hours after sunrise. Similar results have been obtained by the writer and by Deshpande (*12*). The period of anthesis in the pepper is comparatively short. Dehiscence normally follows anthesis very closely, and in some cases is simultaneous with it. Under ordinary growing conditions, the petals begin to wither sometime during the second day and usually drop within 2 or 3 days thereafter. Temperatures lower than 75° F., however, materially slow-up the whole mechanism of anthesis and dehiscence. These findings are substantiated by Shaw and Khan (*20*), who further observed that the mechanism in pepper is delayed on cloudy as well as on cold days.

POLLINATION

The flowers are usually self-pollinated, although some cross-pollination may occur. In fact, since Martin et al. (*18*) have found that *Capsicum* blossoms secrete nectar, which attracts various insects, one is led to believe that cross-pollination takes place more frequently than has generally been supposed. Results of experiments conducted by Erwin (*14*) at Ames and Muscatine, Iowa, show that either self- or cross-pollination may occur. Similar observations have been made in years past at the Georgia Experiment Station.

After the pollen is deposited on the stigma it remains inactive for a short time under all environmental conditions. However, the most rapid and the highest percentage of pollen germination takes place at temperatures ranging from 70° to 85° F. accompanied by a rather high relative humidity. These results are well in accord with those reported by Smith and Cochran (*24*) for the tomato.

Blossoms produced under excessively high temperatures (90° to 100° F.) and low humidity practically always have elongated styles. This elongation occurs well in advance of anther dehiscence, which, in this case, makes self-pollination unlikely. Observations made by Burk (*5*) on the tomato show that length of photoperiod also affects style length, the style being longer under long, than under short-day conditions.

FERTILIZATION

The time that elapses between pollination and fertilization varies with the temperature to which the plants are subjected. Blossoms that were hand-pollinated in the 70° to 80° F. greenhouse and the plants transferred to the 50° to 60° greenhouse produced practically all parthenocarpic fruits. Examination of slides made of some of these styles show that parthenocarpy was not due to a lack of pollen germination. Because of the lower temperature the pollen tubes failed to reach the embryo sac. No tubes longer than 2 mm were found.

The pollen tubes grow down through the tissue of the style. At some time during the growth of the pollen tubes, the generative nucleus divides, forming two microgametes. The pollen tube ex-

tends through the micropyle, passing between the two synergids and comes to rest near the egg. The actual discharge of the male nuclei was not observed because of the deeply staining contents of the pollen tube. One nucleus fuses with the egg to form the embryo while the other unites with the two polar nuclei to form the primary endosperm nucleus. The embryo sac at the time of fertilization, which at a temperature of 70° to 80° F. was 42 hours after pollination, is shown in figure 8, *A*. At no time was fertilization observed earlier than 42 hours after pollination. The time elapsed between pollination and fertilization is somewhat shorter than that found by Smith (*22*, *23*) for tomato and by East and Park (*13*) for *Nicotiana*. These differences may be due to differences in the length of the style in the three genera. The style of the pepper averages only 4.5 mm in length, that of the tomato 6.75 mm, and that of *Nicotiana* even longer. No doubt, too, the various temperatures under which the respective plants were grown influenced the time element in fertilization.

DEVELOPMENT OF THE EMBRYO

Apparently Tognini (*27*) made the earliest study of embryo development in the Solanaceae. His study was not complete, including only a few stages of the development of the embryo in *Atropa belladonna*, *Datura stramonium*, *Physalis edulis* and *Solanum tuberosum*.

Souèges (*26*), working with *Atropa belladonna*, *Datura stramonium*, *Hyoscyamus niger*, *Nicotiana acuminata*, *N. sanguinea*, *N. tabacum*, *Solanum dulcamara*, *S. nigrum*, *S. sisymbriifolium*, and *S. villosum*, has reported the most complete study of the embryogeny of the Solanaceae. He found in all of these species that the typical four-celled embryo has its cells arranged in linear order, and these findings are supported by the work of Smith (*23*) with tomato, Bhaduri (*4*) with *Nicotiana plumbaginifolia*, *Petunia nyctaginiflora*, *Physalis minima*, and *Withania somnifera*, and by the present investigation.

After fertilization, the zygote remains in a state of rest for 24 to 36 hours before division starts (fig. 11, *A*). Development of the zygote begins with an enlargement of the embryo sac, especially in length (fig. 11, *B*; fig. 12, *A*). The first division of the young embryo (fig. 12, *B*) is transverse. The two daughter cells then divide transversely, forming a four-celled linear embryo (fig. 12, *C*). In the pepper each cell of the four-celled embryo gives rise to certain definite portions of the mature embryo, but an exception to this has been observed by Bhaduri (*4*) in *Physalis minima*. In the present study, it was found that the cotyledons are derived from the apical cell; the hypocotyl, the initials of the central cylinder, and the cortex of the root tip from the cell just below it; the root-cap primordium and upper part of the suspensor from the next lower cell; and the remaining part of the suspensor from the basal cell. Further development of the embryo is shown in figure 12, *D-J*. As is noted in figure 12, *E* and *F*, the two apical cells *a* and *b* first divide longitudinally, whereas *c* and *d* divide transversely, thus forming an eight-celled embryo, six cells in length. The next division is periclinal, which gives rise to both an outer and an inner group of cells (fig. 12, *G*). The outer cells form the dermatogen while the inner ones divide transversely, separating the initials of the hypocotyl and the radicle. In figure 12,

J, the dermatogen, the periblem, the periblem initials, and the plerome have differentiated.

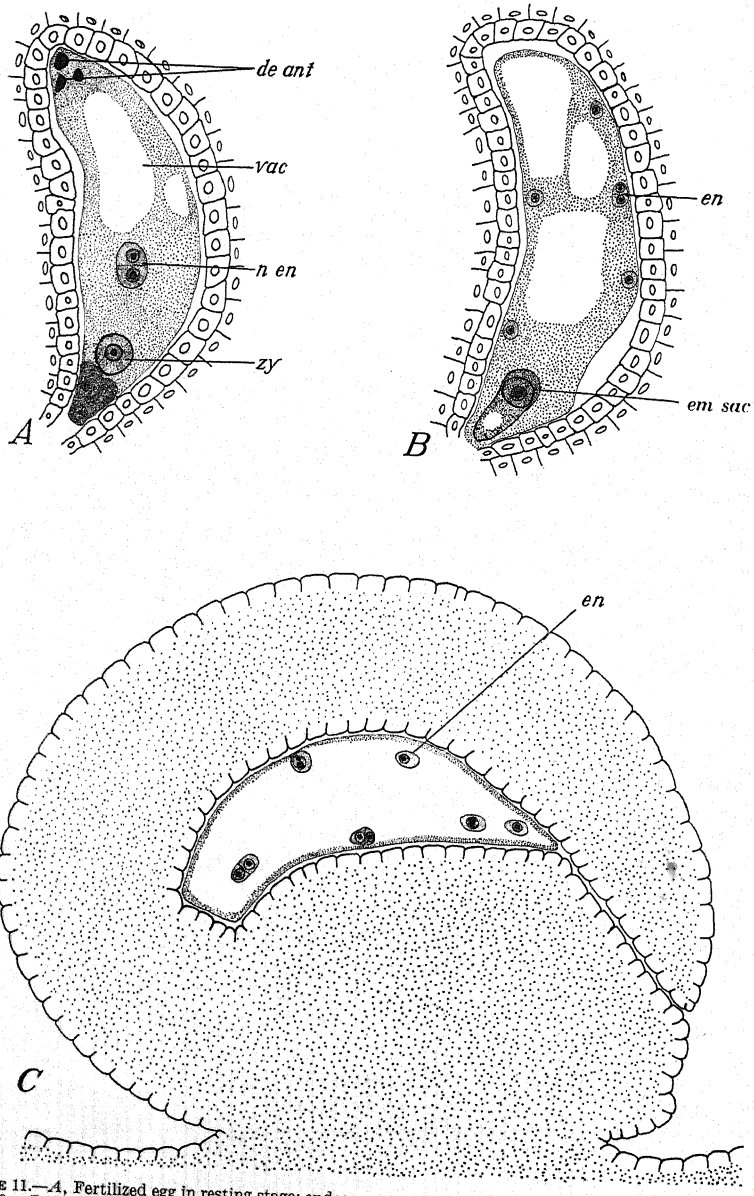


FIGURE 11.—*A*, Fertilized egg in resting stage; endosperm nuclei prior to division; antipodals degenerating. × 950. *B*, Young zygote increasing in size prior to division; endosperm nuclei divided and migrated to periphery of embryo sac. × 950. *C*, Ovule showing endosperm nuclei undergoing division. × 640. *de ant*, Degenerating antipodals; *vac*, vacuole; *n en*, nascent endosperm; *zy*, zygote; *em sac*, embryo sac; *en*, endosperm.

A longitudinal section of the basal tip of the embryo, when the fruit is in the red-ripe stage of maturity shows the root cap, the

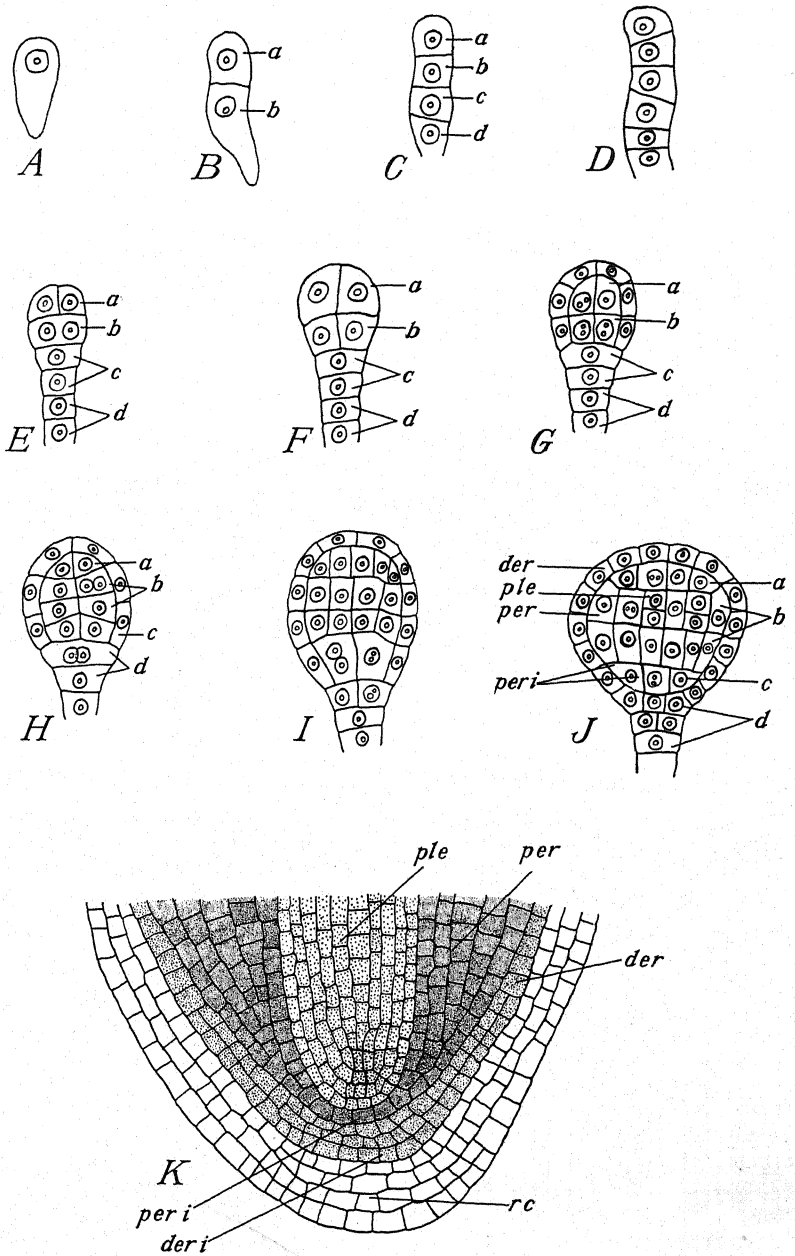


FIGURE 12.—*A*, Young zygote a few hours prior to the first transverse division. × 950. *B–E*, Development of embryo to two, four, six, and eight-nucleate stages respectively. × 950. *F*, Further division of the octant stage. × 950. *G*, Embryo with dermatogen cells cut off in tiers *a* and *b*. × 950. *H*, Embryo showing differentiation of initials of hypocotyl and root tip. × 950. *I*, Further development of embryo as shown in *H*. × 950. *J*, Embryo with dermatogen, periblem, periblem initials, and plerome differentiated. × 950. *K*, Longitudinal section of basal end of a practically mature embryo showing dermatogen, periblem, plerome, periblem initials, dermatogen initials, and root cap. × 440. *der*, Dermatogen; *ple*, plerome; *per*, periblem; *per i*, periblem initials; *der i*, dermatogen initials; *r c*, root cap.

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dermatogen, the periblem, and the plerome (fig. 12, *K*). Further growth of the embryo and the endosperm is shown in figure 13 *A–F*, figure 14, *A–D*, and figure 15, *A–C*.

DEVELOPMENT OF THE ENDOSPERM

The primary endosperm nucleus, formed by the union of the fused polars with the second male nucleus, begins to divide well in advance of that of the zygote (fig. 11, *B*). The nuclei migrate toward the periphery (fig. 11, *B* and *C*), where they continue to divide very rapidly, soon completely filling the embryo sac. With the rapid growth of the embryo, a portion of the endosperm is gradually digested and apparently absorbed. However, this process is so slow that it cannot be readily detected until approximately 20 to 25 days after pollination. Prior to and including this stage of development, both the endosperm and the embryo cells are well filled with reserve food. Evidently the young seedling is able to use this material until it begins to manufacture its own food. The inner epidermal layer of the integument, which in all probability also aids in nourishing the embryo, is easily recognized and persists until the cotyledons are well-differentiated (fig. 15, *A*, *B*). It is the opinion of Souèges (*25*) that the inner epidermal layer secretes diastase, which causes, the central part of the integument to break down and subsequently disappear. After a lapse of 30 days following pollination, the rate of endosperm absorption increases with the increase in size of the embryo. At maturity of the seed, however, a large portion of the endosperm still remains (fig. 15, *C*).

DEVELOPMENT OF THE INTEGUMENT

The integument may be detected during the early developmental stages of the young ovule. Usually it makes its first appearance soon after the differentiation of the archesporial cell (fig. 5, *B*, *C*) and portions of it persist throughout seed maturity. Two days after pollination, the outer epidermal cells of the integument are practically isodiametric (fig. 16, *A*). However, as the fruit and seed mature, the cells elongate radially (fig. 16, *B*). Within 25 days after pollination, heavy thickenings are formed on the lateral walls (fig. 16, *C*). These thickenings continue to grow with the development of the seed, finally reaching their maximum size about 30 days after pollination (fig. 16, *D*). The thickenings are characteristically larger at the base of the walls than at any other point and become smaller as they extend toward the outer wall. These findings are confirmed by the earlier work of Hanausek (*16*), Hartwich (*17*), and Souèges (*25*). Unlike the thickenings in tomato (Souèges (*25*), Smith (*23*), those in pepper extend the entire length of the lateral walls, and a smaller proportion of the integument degenerates in the pepper than in the tomato. As a result of this there are small depressions on the surface of the pepper seed, whereas on the tomato seed there are long hairs. In cross section, the thickenings are irregular and wavy in outline (fig. 16, *E*).

SUMMARY

In the development of the pepper flower, the first primordial whorl to appear is that of the sepals. The remaining three whorls of organs appear in the order of petals, stamens, and carpels.

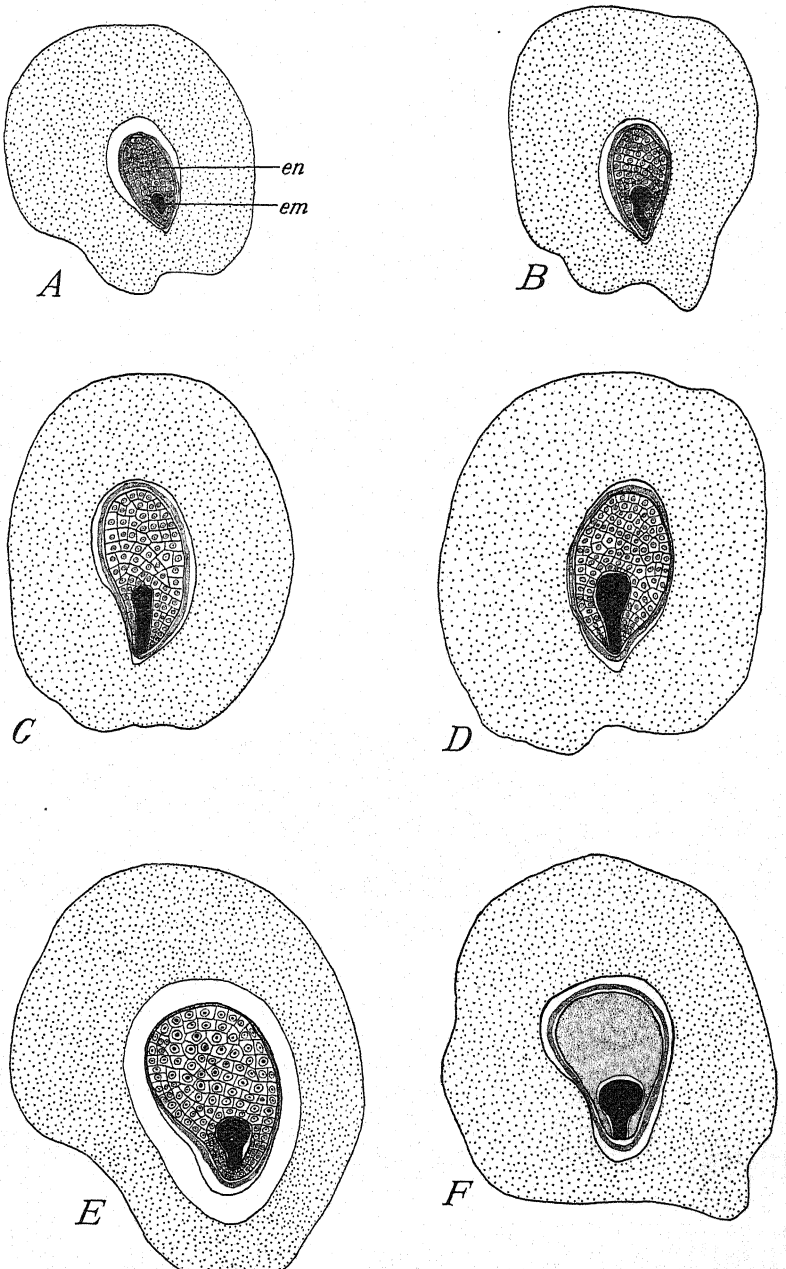


FIGURE 13.—*A–F*, Relative growth of embryo and endosperm; *A*, longitudinal section of young two-celled embryo 72 hours after pollination; *B*, *C*, *D*, *E*, *F*, ovule 5, 7, 9, 11, and 13 days respectively after pollination; *en*, endosperm; *em*, embryo. All × 225.

The young ovule arises from the placental tissue as a small erect protuberance, but subsequently becomes anatropous in form. Early in the development of the ovule, a single hypodermal cell of the

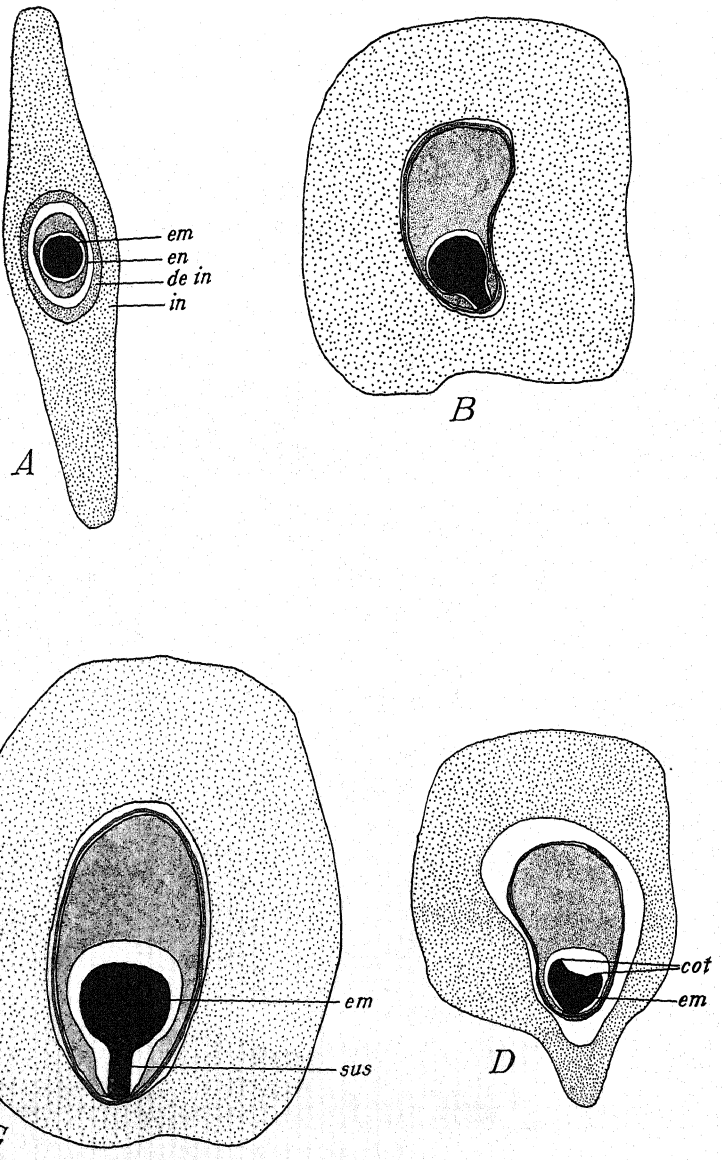


FIGURE 14.—*A*, Transverse section of young ovule showing embryo 13 days after pollination; *B*, longitudinal section of young ovule 15 days after pollination; *C*, ovule 17 days after pollination; *D*, ovule 21 days after pollination, showing initiation of cotyledons; *em*, embryo; *en*, endosperm; *de in*, degenerating portion of integument; *in*, integument; *sus*, suspensor; *cot*, cotyledons. All × 225.

nucellus is differentiated as the archesporial cell, which in this case functions as the macrospore mother cell. By two divisions, the macrospore mother cell forms a linear row of four macrospores of

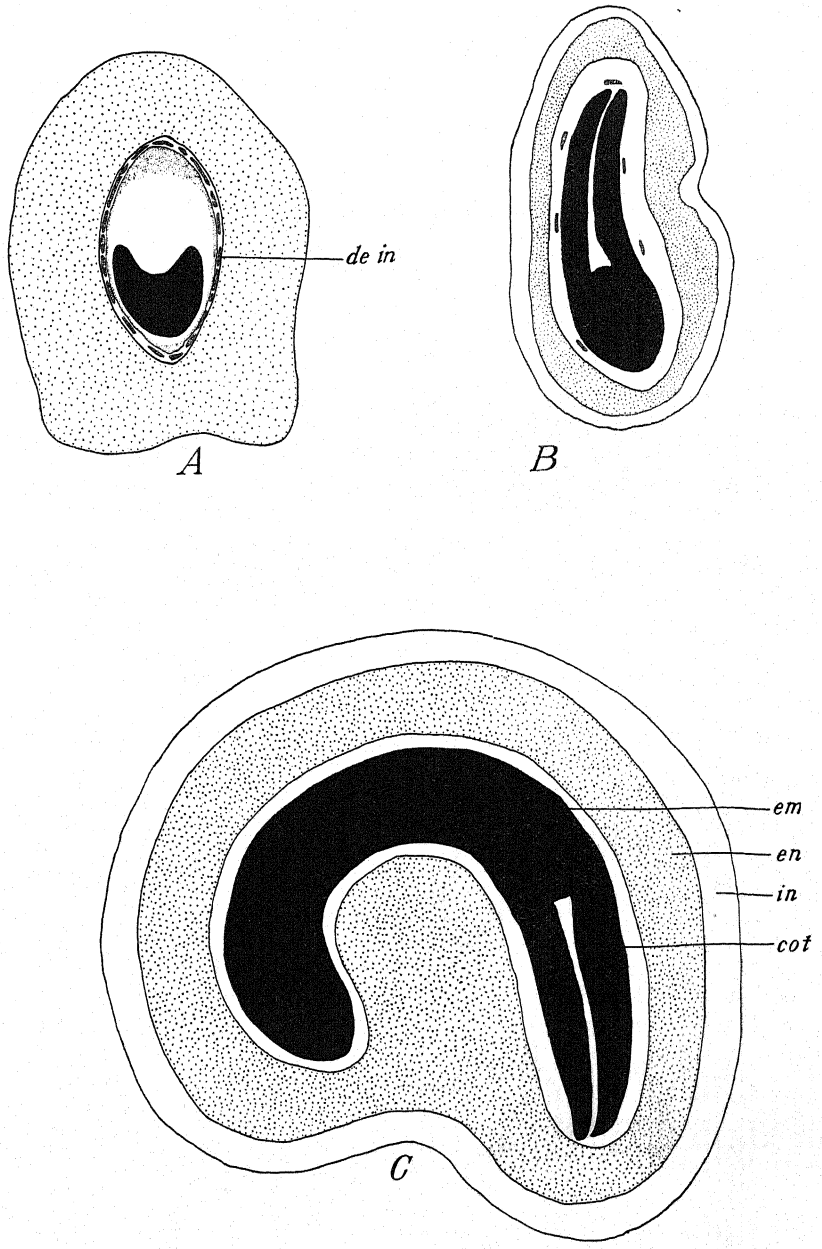


FIGURE 15.—*A*. Longitudinal section of ovule 24 days after pollination, showing development of cotyledons and degeneration portion of integument; *B*, ovule 30 days after pollination, showing further development of cotyledons; inner layer of integument almost completely degenerated; *C*, ovule 40 days after pollination, showing mature embryo; *de in*, degenerating portion of integument; *em*, embryo; *en*, endosperm; *in*, integument; *cot*, cotyledons. All × 225.

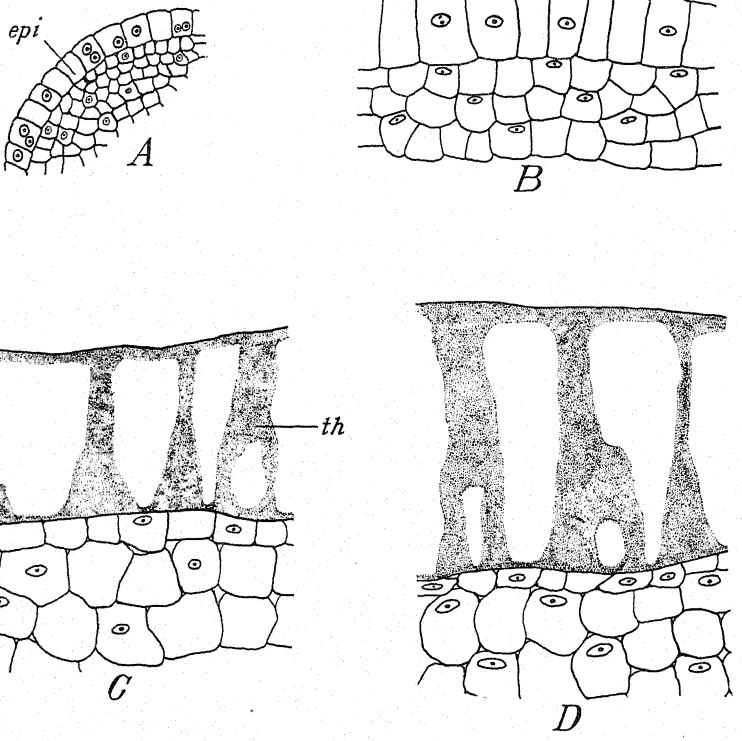


FIGURE 16.—*A*, Outer epidermis and adjacent cells of integument 2 days after pollination, × 750; *B*, outer epidermis of integument showing radial elongation of epidermal cells, × 750; *C*, longitudinal section of epidermis of integument, showing thickenings on lateral walls 25 days after pollination, × 950; *D*, longitudinal section of epidermis of integument, showing thickenings on lateral walls 30 days after pollination, × 950; *E*, transverse section near base of thickenings 36 days after pollination, × 950; *epi*, epidermis; *th*, thickenings.

approximately the same size. The three macropsores nearest the micropyle soon degenerate, leaving the chalazal one which enlarges and becomes functional. By three successive divisions of the functional macrospore, the typical eight-nucleate or mature embryo sac is formed. Two of the nuclei take a position near the center of the sac and become the polars; three migrate to the chalazal end of the sac and become the nuclei of the antipodal cells; the three remaining nuclei take a position near the micropylar end of the sac; the two more pear-shaped ones become the synergids, and the remaining one the egg. The antipodal cells subsequently completely degenerate as do those of the nucellus.

Pollen mother cells arise as a result of periclinal division of the hypodermal archesporial cells that form within the young anther. Each pollen mother cell, by two divisions, forms a tetrad of microspores. The sister cell of the pollen mother cell gives rise to the tapetum, the middle layer, and the endothecium. Mature pollen grains are two-nucleate, containing a spherical generative nucleus and a spherical vegetative nucleus. The generative cell divides to form two male gametes within the growing pollen tube before it reaches the embryo sac. One gamete nucleus unites with the polar nuclei to form the endosperm while the other fuses with the egg to give rise to the embryo.

The higher the temperature to which plants are exposed, up to 90° to 100° F., the more quickly anthesis occurs. Anthesis takes place 10 days earlier under normal-day than under long-day conditions. Dehiscence normally follows anthesis very closely.

The pepper flower is usually self-pollinated.

The time that elapses between pollination and fertilization varies with the temperature to which the blossom is subjected. Fertilization was first observed in the 70° to 80° F. greenhouse 42 hours after pollination.

The zygote does not begin to divide until 24 to 36 hours after fertilization. The first division of the zygote is transverse. The two resulting cells then divide transversely, thus forming a four-celled embryo with its cells arranged in linear order. This arrangement is typical of most members of the Solanaceae. Each cell of the four-celled embryo gives rise to certain definite portions of the mature embryo. The cotyledons are derived from the apical cell; the hypocotyl, the initials of the central cylinder, and the cortex of the root tip from the cell just below it; the root-cap primordium and the upper portion of the suspensor from the next lower cell; and the remaining part of the suspensor from the basal cell.

The endosperm nuclei begin division well in advance of those of the embryo and they soon fill the entire embryo sac. The endosperm is gradually digested and absorbed by the embryo; however, at maturity a large portion of the endosperm still remains.

The outer epidermal cells of the integument are at first isodiametric. As the fruit and seed mature, however, these cells elongate radially as a result of degeneration of tangential walls, and soon thereafter heavy thickenings form at the base of the lateral walls. The thickenings are larger at the base of the walls and become smaller as they extend toward the outer wall.

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STUDIES ON LONGEVITY AND PRODUCTIVITY IN TRICHOGRAMMA EVANESCENS[1]

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INTRODUCTION

During recent years entomologists have expended considerable time and ingenuity in an effort to utilize successfully the chalcid egg parasite *Trichogramma* in the biological control of certain insect pests. Since the results of these efforts have not been altogether encouraging, it is hoped that the present study of the influence of certain environmental factors upon the longevity and the productivity of the adult parasites may help to elucidate the problems involved. To date, the only comprehensive work along this line is that published by Schulze (*27*).[3] The results of her work are discussed in connection with the results of the present experiments.

The present paper records the effects of feeding, absence of host eggs, mating, superparasitism, diffused light, temperature, and moisture upon the longevity and productivity of *Trichogramma evanescens* Westwood.

EXPERIMENTAL PROCEDURE

The Angoumois grain moth, *Sitotroga cerealella* (Oliv.), was chosen as the host in these experiments largely because it is the species most commonly used in mass rearing for pest control. The host insects were reared in shelled corn, and in order to procure the moth eggs for parasitism by *Trichogramma*, several hundred individuals were collected in a quart jar by means of a small suction trap. In this crowded condition, the females readily deposited their eggs, which were easily collected simply by shaking them out through a screen lid (22 meshes to the inch). After the eggs had been cleaned of debris, they were fastened to small bits of paper by means of dilute gum arabic, and each egg paper was labeled with the date, the time, and the number of the vial in which the female parasite being tested was confined. The eggs were then placed in the vial with the parasite for parasitism.

The parasites were reared in small desiccators at 30°, 25°, 20°, 15°, and 10° C. in cabinets regulated by toluene-mercury thermostats, the temperatures of which did not vary significantly more than one-half degree centigrade.

The moisure conditions in the desiccators were controlled by means of ample volumes of sulphuric acid solutions, which were changed frequently and were exposed to the air only as long as was absolutely

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[3] Reference is made by number (italic) to Literature Cited, p. 437.

necessary in making the observations. It is believed that the time required for the atmosphere in the small rearing vials to arrive at an equilibrium with the atmosphere in the desiccators was not significantly long, since the vials were out of the desiccators only a very few minutes either once or twice daily, the desiccators themselves were small, and the tops of the vials were never more than 2½ inches above the surface of the liquid. The desiccators, unless otherwise stated, were covered with a heavy coating of black paint to eliminate any possible effect of light variation.

In testing the effects of temperature, an attempt was made to eliminate moisture as a variable factor, i. e., to maintain moisture conditions such that the total evaporation during the period of exposure would be about the same at each temperature. Mellanby (*18*) points out that this state of affairs is realized only when the products of the exposure times and the saturation deficiencies (expressed in millimeters of mercury) are constant. Such constant products were attained very well at the three higher temperatures used (30°, 25°, and 20° C., where the products were 52.5, 50.0, and 50.0, respectively, for development, and 30.0, 30.0, and 26.2, respectively, for adult longevity), but at 15° and 10°, the use of a sufficiently low saturation deficiency to produce a constant product was deliberately avoided for fear of the development of mold, and, as a consequence, the total evaporation was somewhat greater at the two lower temperatures than at the other temperatures.

The experimental procedure was so planned and controlled that the following facts could be recorded: The conditions under which each immature and adult parasite was reared; the time of emergence of the parent parasites from the host eggs; the time that these recently emerged males and females were placed together; the time during which each batch of fresh host eggs was parasitized; the number of host eggs parasitized in each batch; the sex ratio of the emerged progeny; and the time of death of each parent parasite. (The parasites were considered to be dead when they were no longer able to walk.)

The actual procedure in setting up an experiment was as follows: Fresh host eggs were carefully selected under a binocular dissecting microscope (30×) and transferred individually onto graph paper (lined to one-tenth of an inch), to which they were fastened with dilute gum arabic, according to the method of Salt (*23*). Batches of 100 eggs so arranged were then placed for parasitism in 3- or 4-dram vials stoppered with corks containing ¼-inch perforations covered with silk bolting cloth. These vials were maintained under controlled conditions until the progeny began to emerge. Within 12 hours (24 hours when reared at 20° C. or lower) after the first emerging progeny appeared, the egg papers were removed and each individual parasitized host egg was cut away from the others and dropped into a separate ½-dram vial stoppered with a cork containing a ⅛-inch perforation covered with silk bolting cloth. These vials were then replaced immediately in the original condition, where the parasites completed their development, each vial being checked at intervals of 12 or 24 hours for the emerged parasite. On emergence of the parasite, the vial was emptied of its host-egg paper and numbered. The parasites were then fed, paired, given host eggs, etc., as desired and placed under the environmental conditions in which it was intended that the adults should live and die.

Each 12 or 24 hours until death, fresh moth eggs were presented to each female for parasitism. At the same time, the previous paper of eggs was removed and placed at a uniformly favorable condition of 25° C. and 5-mm saturating deficiency (hereafter referred to as the "standard condition") for the incubation of the contained parasites. In order to make sure that the maximum capabilities of the females under the particular conditions were being expressed, many more host eggs were presented in each case than could possibly be parasitized.

After about 2 weeks (ample time for the parasite progeny to emerge and die) the egg papers were removed from the incubation vials and the parasitized host eggs on each paper counted. This was accomplished readily because of the fact that parasitized *Sitotroga* eggs invariably turn black. The number of blackened host eggs was taken to represent the productivity of the female parasites. This being the case, it should be mentioned that in the writer's experience, *Sitotroga* eggs turn black only when parasitized. In several thousand black host eggs dissected, all contained parasites. Furthermore, it is fairly safe to assume that each blackened host egg contained only 1 parasite, since only 5 of 520 (0.96%) parasitized *Sitotroga* eggs cleared in lactophenol solution contained two developing parasites, and Salt (*24*) has shown that *T. evanescens* definitely avoids superparasitism—especially when an abundance of host material is present. It is true, however, that some of the parasite progeny deposited in host eggs may have died, for some reason or other, before they reached the late larval or early pupal stage (at which time, apparently, they cause the coloring of the host egg) and therefore failed to leave visible evidence of their having been produced. The female parasites received credit in these experiments, therefore, for only the number of their progeny that completed 2 or 3 days of development under the standard condition.

At the same time that the parasitized host eggs were counted, the emerged parasite progeny were removed to a drop of clear mineral oil, where they could be manipulated conveniently under a microscope and the sex determined. The sex ratio in *Trichogramma* is a convenient indicator of whether or not a particular female has been impregnated, since virgin females produce only male offspring. Thus the partical or complete impotence of the male is reflected in an abnormally low sex ratio.

LONGEVITY AND PRODUCTIVITY AT THE STANDARD CONDITION (25° C. AND 5-MM SATURATION DEFICIENCY)

The insects considered in this section were kept at the standard condition throughout both their developmental and adult periods. The data on the average longevity of isolated pairs of honey-fed males and females and the number of progeny produced by them when they were given fresh host eggs in which to oviposit immediately after pairing and at regular half-day intervals thereafter until death, are summarized in table 1.

The figure 66.1 ± 2.5 for the number of progeny per female is considerably larger than that given by Schulze (*27*), who found a mean of 43.2 parasitized *Ephestia* eggs per female. This discrepancy in results may be due to a difference in method, host, or strain of parasites.

TABLE 1.—*Productivity (host eggs turning black) and longevity of fed pairs at 25° C. and 5-mm saturation deficiency*

Item	N[1]	Range	Mean ± S. E.	C. V.
Progeny.....number..	70	15–114	66. 1±2. 5	31. 8
♀ longevity.....days..	71	1–13	6. 3± . 3	46. 6
♂ longevity.....do....	63	1–9. 5	5. 7± . 2	30. 3

[1] Number of parasites tested.

Bowen (*6*) reared what is probably *Trichogramma evanescens* (see Flanders (*11*)), at room temperature (75°–80° F.) in bagworm eggs (*Thyridopteryx ephemeraeformis* (Haw.)) and found that 42 females averaged 37.0±2.1 progeny, a figure that approximates that given by Schulze. The difference between this figure and that given in table 1 is very probably due also to the differences in host and method.

The distribution of the egg laying through the life of the female is indicated in figure 1.

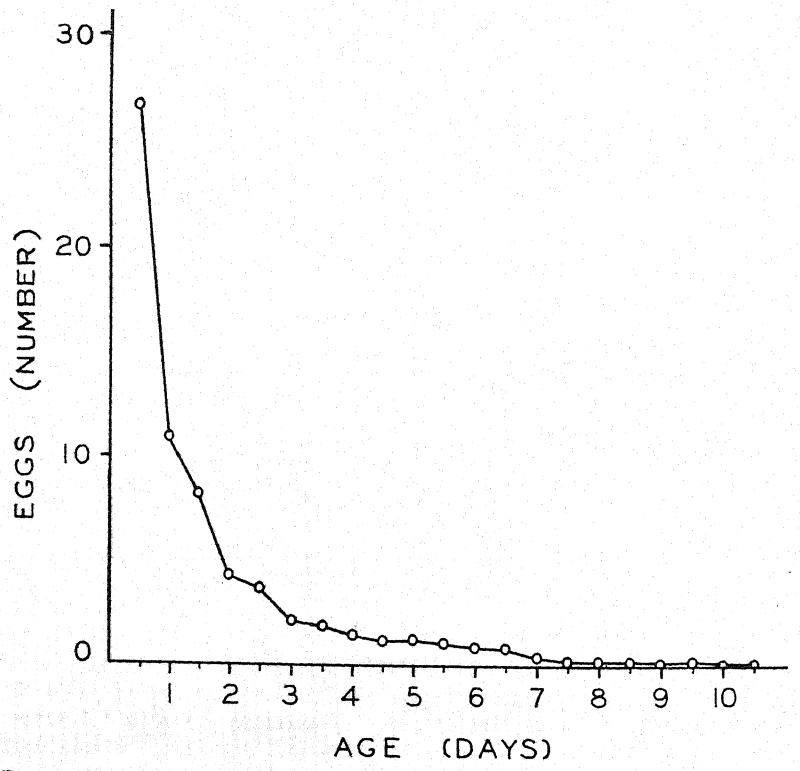


FIGURE 1.—Eggs laid as related to age of adult when kept at 25° C. and 5-mm saturation deficiency.

It will be noticed that almost half (26.75 eggs or 40.5 percent) of the total number of eggs is produced during the first 12 hours of the female's life. This curve differs from the one given by Schulze in that it indicates no rise in the number of progeny produced after the fifth day.

The longevity of mated and fed females reared in *Sitotroga* eggs at 25° C. is 6.3 ± 0.3, with a range of 1 to 13 days. Schulze gives the figure as 13 days, but presents none of the data used in arriving at that figure. Bowen gives the figure as 5.17 ± 0.29 days based upon 42 females reared in *Ephestia* eggs. The writer found that the longevity of paired, fed males is 5.7 ± 0.2 days, not significantly different from the figure for the females. Most of the males die between the fifth and seventh days after emergence however, whereas death of the females is more evenly distributed between the extremes of the life span. This greater spread in length of life of the females is also shown by their coefficient of variation (table 1).

The sex ratio of 1,788 progeny from females paired with males is 0.66 female, the ratio remaining about constant throughout the period of production.

When the longevity of the mated females is correlated with their egg production, a significantly high correlation coefficient of $+0.639 \pm 0.071$ is obtained. This is explained best by assuming that the longer lived females are fundamentally capable of greater egg production, and not merely that they have more time in which to lay eggs. This must be true since during the first 2 days 76.0 percent of the eggs are produced, at the end of which time 92.9 percent of all the females are still living. In other words, high total egg production is correlated with high production in the initial period of egg laying. Thus the 36 highest producers (averaging 82.2 total progeny per female) produced 57.9 progeny the first 2 days of production; while the lowest 34 producers (averaging 49.1 total progeny per female) produced only 40.8 progeny during the first 2 days.

INFLUENCE OF DIFFERENT FACTORS ON LONGEVITY AND PRODUCTIVITY

EFFECTS OF FEEDING

At the standard condition, those adult parasites that were fed on dilute honey placed in a thin streak on the inside of each vial, lived significantly longer than starved parasites. This was true of isolated females, isolated males, male-female pairs, and female-female pairs—whether host eggs were available or not. In all cases, moreover, fed females produced significantly more progeny than starved females. The data for virgin females in table 2 are representative of the results obtained in all the combinations tried.

TABLE 2.—*Productivity (black host eggs) and longevity of fed and unfed virgin females*

Item	Fed virgins		Unfed virgins		Fed virgins minus unfed virgins	
	N	Mean± S. E.	*N*	Mean± S. E.	Difference± S. E.	Difference / S. E. difference
Progeny, number	95	81.5±2.2	79	60.8±1.7	20.7±2.8	7.4
Longevity, days	93	6.4±.3	79	3.3±.1	3.1±.3	10.3

These data confirm, in general, the findings of Schulze (*27*), Peterson (*21*), and List and Davis (*15*) and indicate that feeding the parasites before liberation in the field will heighten their effectiveness by increasing both their longevity and their productivity.

EFFECTS OF WITHHOLDING HOST EGGS

In these experiments, conducted at the standard condition, host eggs were withheld from fed virgin females for 48 hours, for 72 hours, and for the entire duration of the life of the female. In table 3 longevity and productivity of the females under each of these conditions are compared with those of females that were kept in the presence of fresh host eggs throughout their lives.

It is evident that virgin females live longer in the presence of host eggs than in their complete absence. This is in accordance with the findings of Hase (*13*), who suggests that the longer life in the presence of host eggs may be due to the habit of the female of feeding on the minute droplet of liquid exuding from the puncture in the chorion of the host egg from which she has just withdrawn her ovipositor, and may explain in part the pronounced effectiveness of the parasites in the field in times of host abundance and their utter ineffectiveness in times of moderate or meager host abundance, since the finding of a readily available host egg by the female parasite in itself increases her effectiveness by lengthening her life.

On the other hand, if the host eggs are withheld from virgin females for 48 or for 72 hours, longevity is unaffected, but productivity is significantly reduced. Thus the egg-laying ability of females that are prevented from ovipositing diminishes with increasing age, even though half or two-thirds of their life span remains after the host eggs are given them. Schulze (*27*) found that if females were deprived of host eggs for as long as 5 days, their longevity was not reduced. Since, however, the productivity of females diminishes rapidly after emergence, the advantage of liberating young parasites in field control measures is apparent.

TABLE 3.—*Productivity (black host eggs) and longevity of virgin females given host eggs at once compared with those of females from which host eggs were withheld for various lengths of time*

Treatment and factors observed	*N*	Mean± S. E.	Difference± S. E.	Difference / S. E. difference
Host eggs given at once:				
Progeny.....number..	95	81. 5±2. 2 (1)	.....	.....
Longevity.....days..	93	6. 4±. 3 (1)	.....	.....
Host eggs withheld 48 hours:				
Progeny.....number..	30	54. 7±4. 1 (2)	[1] 26. 8±4. 7	5. 7
Longevity.....days..	30	6. 3±. 6 (2)	[1] . 1±. 7	. 1
Host eggs withheld 72 hours:				
Progeny.....number..	23	44. 7±4. 2 (3)	[2] 36. 8±4. 8	7. 7
Longevity.....days..	23	7. 3±. 5 (3)	[2] −. 9±. 6	1. 5
Host eggs withheld entirely:				
No progeny.....				
Longevity.....days..	31	4. 3±. 3 (4)	[3] 2. 1±. 4	5. 2

[1] (1 minus 2). [2] (1 minus 3). [3] (1 minus 4).

EFFECTS OF PAIRING

In these experiments the fed females were given host eggs immediately upon emergence but were individually isolated for 24 hours or

for their entire lives at the standard condition. In table 4 the longevity and productivity of these females are compared with those of females kept in the presence of males from the time of emergence until death.

TABLE 4.—*Productivity (black host eggs) and longevity of females paired with males immediately compared with those of females whose pairing was delayed*

Treatment and factors observed	*N*	Mean±S. E.	Difference± S. E.	Difference / S. E. difference
Pairing immediate:				
Progeny.....number..	70	66. 1±2. 5 (1)	.....	.....
Longevity.....days..	71	6. 3± . 3 (1)	.....	.....
Pairing delayed 24 hours:				
Progeny.....number..	25	66. 8±4. 2 (2)	[1] 0. 7±4. 9	0. 1
Longevity.....days..	25	7. 0± . 6 (2)	[1]. 7±. 7	1. 0
Pairing entirely prevented:				
Progeny.....number..	95	81. 5±2. 2 (3)	[2] 15. 4±3. 3	4. 7
Longevity.....days..	93	6. 4±. 3 (3)	[2]. 1±. 4	. 2

[1] 2 minus 1. [2] 3 minus 1.

The data in table 4 indicate that, although the longevity of the females is unaffected by pairing, there is a definite increase in the number of progeny when the females remain virgin throughout their lives. Evidently the presence of the male in some way inhibits oviposition. That the mere presence of another insect, rather than the disturbance caused by the act of copulation itself, is responsible for the lower productivity of paired females is suggested by the fact that the presence of a second female has the same effect as the presence of a male ($N=30$; average productivity in female-female pairs= 67.1 ± 2.5).

Schulze (*27*) also found that unimpregnated females produced more progeny than impregnated females (48.4:43.2), and Peterson (*21*) states that unfertilized females produced "as numerous" progeny as did the fertilized. On the other hand, Hanson and Ferris (*12*) found that mated females of *Drosophila melanogaster* Meig., a species that does not reproduce parthenogenetically, lay from 1.6 to 2.0 times more eggs than do virgins, the presence of the male stimulating, rather than inhibiting, oviposition.

Females given host eggs immediately upon emergence but denied the opportunity to mate until they are 24 hours old, do not differ either in longevity or productivity from females mated immediately upon emergence. Males, however, that are denied the opportunity to mate until they are 48 hours old live significantly longer than males paired immediately upon emergence (mean=6.6 days as against 5.9 days; difference S. E. difference=2.9). That male potency is unaffected by this delay in mating is indicated by the fact that a normal proportion of the progeny produced immediately after pairing is made up of females (0.68 female in 1,112 progeny).

EFFECTS OF SUPERPARASITISM

The data discussed in this section refer to fed pairs, each consisting of a male and a female parasite reared in the same *Sitotroga* egg under the standard condition. The data were assembled from records taken on scattered cases of superparasitism encountered in the course of several experiments, and are presented in table 5.

TABLE 5.—*Productivity (black host eggs) and longevity of pairs reared to maturity in separate host eggs compared with those of pairs reared in the same host egg*

Item	Pairs from separate host eggs		Pairs from the same host egg		Pairs from separate host eggs minus pairs from the same host egg	
	N	Mean±S. E.	*N*	Mean±S. E.	Difference± S. E.	Difference / S. E. difference
Progeny.....number..	70	66. 1±2. 5	19	51. 9±3. 2	14. 2±4. 1	3. 5
♀longevity.....days..	71	6. 3± . 3	20	6. 6± . 5	−. 3± . 6	. 5
♂longevity.....do....	63	5. 7± . 2	17	3. 4± . 4	2. 3± . 4	5. 7

While the number of cases is small, it is evident from table 5 that crowding of the immature stages of both male and female into the same host egg significantly reduces the number of progeny. The longevity of the male is reduced while that of the female is unaffected. In all except two of these cases of superparasitism, the male was very small and wingless, while the female, although definitely smaller than her normal sisters, was fully winged and considerably larger than the male. In superparasitized *Sitotroga* eggs the female is apparently able to thrive at the expense of the male. The dwarf males, however, are sexually potent, as is indicated by a very high proportion of female progeny produced by these pairs (sex ratio is 0.82 female in 495 progeny examined).

Paralleling these findings on *T. evanescens* are the data of Alpatov (*1*), who found that undersized females of *Drosophila melanogaster* (larvae taken from their food prematurely) lived just as long as normal females and (*2*) that underfeeding reduced productivity. Norris (*20*) found also that the number of eggs produced by *Ephestia kuehniella* Zell. was reduced by rearing on an abnormally small quantity of food.

EFFECTS OF DIFFUSED LIGHT

In these experiments, fed virgin females were exposed to a constant diffused light throughout their adult lives at the standard condition. The source of the light was an ordinary Mazda bulb suspended 9 inches above the double-glass top of the temperature cabinet, and the intensity, as measured on a Western Electric photometer, was about 75 foot-candles.

TABLE 6.—*Effect of diffused light upon the productivity (black host eggs) and longevity of isolated virgin females*

Item	Virgins kept in darkness		Virgins kept in light		Virgins in darkness minus virgins in light	
	N	Mean±S. E.	*N*	Mean±S. E.	Difference± S. E.	Difference / S. E. difference
Progeny.....number..	95	81. 5±2. 2	64	76. 8±2. 9	4. 7±3. 6	1. 3
Longevity.....days..	93	6. 4± . 3	66	7. 2± . 3	−. 8± . 4	2. 0

It will be seen from table 6 that there is no significant difference between the number of progeny produced by virgins kept in the light

and those kept in the dark, but the longevity of females in the lighted desiccators is slightly greater than that of females kept in the dark. This is contrary to what other workers have found. Thus, Peterson (*21, p. 8*) states: "There is strong evidence in the writer's investigations to show that more eggs are parasitized on bright days than on dull cloudy ones. In artificial production, the correct intensity of light is important." And Flanders (*9*) writes: "Light intensity appears to be the dominating influence in the activity of *Trichogramma*." It may well be that the effects of light and temperature were not differentiated by these observers, but it is also possible that differences in the intensity of the light and its spectral composition may account for the discrepancies between the observations of Peterson and Flanders and those indicated in table 6. The present limited observations, however, suggest that light may be of less importance in artificial rearing than has been supposed.

EFFECTS OF TEMPERATURE

Effects of Temperature Acting Upon the Adults

In these experiments, the parasites were all reared from egg to maturity at the standard condition of 25° C. and 5-mm saturation deficiency. Immediately after emergence the adults were fed, paired, and placed at the temperatures indicated in table 7, where they remained for the duration of their lives.

In making the daily observations on the parasites kept at 20°, 15°, and 10° C., the exposure to room temperature averaged somewhat less than 2½ minutes each day, whereas in the experiments run at 25° and 30°, the morning and evening exposure to room temperature totaled less than 10 minutes daily.

Table 7.—*Productivity (black host eggs) and longevity of pairs at successive 5° temperature intervals*

Treatment and factors observed [1]	*N*	Mean±S. E.	Difference± S. E.	Difference / S. E. difference
30° (7.5 mm):				
Progeny, number	90	63.2±1.6		
♀ longevity, days	90	4.1± .1		
♂ longevity, do	87	3.6± .1		
25° (5.0 mm):				
Progeny, number	70	63.1±2.5	[2] 2.9±3.0	1.0
♀ longevity, days	71	6.3± .3	[2] 2.2± .3	7.3
♂ longevity, do	63	5.7± .2	[2] 2.1± .2	10.5
20° (2.5 mm):				
Progeny, number	120	61.9±1.4	[3] −4.2±2.9	1.4
♀ longevity, days	119	11.2± .3	[3] 4.9± .4	12.2
♂ longevity, do	119	10.1± .2	[3] 4.4± .3	14.7
15° (2.5 mm):				
Progeny, number	68	46.3±2.4	[4] −15.6±2.8	5.6
♀ longevity, days	68	15.2± .6	[4] 4.0± .7	5.7
♂ longevity, do	68	15.1± .7	[4] 5.0± .7	7.1
10° (2.5 mm):				
Progeny, number	60	1.2−		
♀ longevity, days	60	17.5± .7	[5] 2.3± .9	2.5
♂ longevity, do	59	14.7± .6	[5] −.4± .9	.4

[1] Temperature in degrees centigrade. Saturation deficiencies in millimeters of mercury.
[2] 25° minus 30°.
[3] 20° minus 25°.
[4] 15° minus 20°.
[5] 10° minus 15°.

It will be noticed from table 7 that the longevity of the adults is inversely related to the temperature down to 15° C., below which life is scarcely, if at all, prolonged. The number of progeny produced holds a fairly constant level from 30° down to 20°, but drops off sharply below this. From general work on temperature optima, however, it seems likely that a more or less definite optimum point for egg production in *Trichogramma* lies somewhere between 30° and 25°. It would appear that 10° is about the lowest temperature at which the mechanism of oviposition in *T. evanescens* can function.

Although Schulze (*27*) does not state the number of cases upon which her data are based nor the humidity conditions to which the parasites were exposed, she gives the average longevity of *Trichogramma* at 25° C. as 13 days, and at 29.5° as 5 days. Both of these figures are considerably larger than the corresponding figures in table 7.

Schulze's figures for productivity at different temperatures (10.0 at 15° C. and 34.0 at 29.7°) are much lower than those in table 7. This is probably due to the fact that she incubated the parasitized host eggs at the temperature at which parasitism took place. Thus she measured the effects of adverse temperatures upon both egg production and egg development rather than upon egg production alone. She used *Ephestia* eggs rather than *Sitotroga* eggs as host material.

Data are given by van Steenburgh (*28*) for 12 isolated females of a "gray strain" (probably *T. evanescens*) kept at each of four temperatures roughly comparable to those given in table 7. The wide discrepancies in results would certainly seem to indicate that some gross difference in species or method must exist.

The general fact illustrated here of the increase in adult longevity in insects with a decrease in the temperature to which the adults are exposed was pointed out by Pictet as early as 1913 (cited by Uvarov (*30*) and by Baumberger (*4*) in 1914. This same relationship was found to be true for *Drosophila* by Loeb and Northrop (*16*, *17*) and Alpatov and Pearl (*3*), and for several species of insects by Janisch (*14*).

From table 7 it will be noticed that at all temperatures the females live longer than the males (ratios of mean differences to the standard errors of the differences at 30° and 20° C. are 3.3 and 3.0, respectively); but at 25° and 15° the differences are statistically insignificant. It seems likely that the general rule is a greater longevity in the females than in the males, even though the data at 25° and 15° are not convincing on this point.

The existence of an optimum temperature for egg production in insects has been recognized for some time. This is indicated, for example, in the work of Ewing (*8*) on *Aphis avenae* Fab.; Titschak (*29*) on *Tineola biselliella* (Hum.); Schubert (*26*) on *Piesma quadrata* Fieb.; and Menusan (*19*) on *Acanthocelides obtectus* (Say).

As for the distribution of the egg laying throughout the lives of the females included in the present study, it may be said that the number of progeny produced on the first day varies directly with the temperature, the oviposition tending to stretch out over a longer period at the lower temperatures.

Table 8 indicates the relative numbers of females produced by parasites kept at the different temperatures. It would appear that any variation from 25° C. lowers the relative number of females. The explanation probably lines in a differential stimulation or inhibition of copulation or oviposition rate.

TABLE 8.—*Sex ratios of the progeny of pairs placed at different temperatures immediately after emergence*

Temperature (° C.)	♀/total	Total counted
30	0.43	4,232
25	.66	1,788
20	.52	5,520
15	.37	2,603
10	.28	57

Since the insects live much longer at 20° than at 30° C. (and length of life is certainly a variable upon which the ability of the parasite to find its hosts is at least partially dependent) and since the difference in productivity at the two temperatures is relatively small, there would seem to be some advantage in liberating the parasites for inundative field control (Flanders (*10*)) when the temperature is between 20° and 25° rather than 25° to 30° or higher.

EFFECTS OF TEMPERATURE ACTING UPON THE IMMATURE STAGES IN THE HOST EGGS

In the experiments treated in this section, the *Trichogramma* were reared to maturity at 30°, 25°, 20°, or 15° C. but were allowed to spend their adult lives at the standard condition of 25° and 5-mm saturation deficiency. Thus the influence of these several temperatures upon the development of the sexual functions and products was tested at a single temperature. The effects of a temperature of 10° acting upon the immature forms could not be determined, since about 900 parasitized host eggs failed to produce a single adult at this temperature.

It will be noticed in table 9 that the longevity of both males and females at the standard condition is reduced as the temperature at which the immature stages are kept is lowered. On the other hand, rearing at either higher or lower temperatures than 25° C. reduces the subsequent productivity at the standard condition.

TABLE 9.—*Productivity (black host eggs) and longevity at 25° C. of pairs reared to maturity at different temperatures*

Treatment and factors observed [1]	*N*	Mean±S. E.	Difference±S. E.	Difference / S. E. difference
30° (7.5 mm):				
Progeny ..... number	39	53.5±4.3	.....	.....
♀ longevity ..... days	38	7.9± .3	.....	.....
♂ longevity ..... do	37	6.8± .3	.....	.....
25° (5.0 mm):				
Progeny ..... number	70	66.1±2.5	[2] −12.6±4.9	2.6
♀ longevity ..... days	71	6.3± .3	[2] 1.6± .4	4.0
♂ longevity ..... do	63	5.7± .2	[2] 1.1± .4	2.7
20° (2.5 mm):				
Progeny ..... number	65	57.9±1.8	[3] 8.2±3.1	2.6
♀ longevity ..... days	65	6.2± .3	[3] 0.1± .4	.2
♂ longevity ..... do	67	5.6± .2	[3] 0.1± .3	.3
15° (2.5 mm):				
Progeny ..... number	113	38.3±1.1	[4] 19.6±2.1	9.3
♀ longevity ..... days	113	4.7± .2	[4] 1.5± .4	3.7
♂ longevity ..... do	110	4.5± .1	[4] 1.1± .2	5.5

[1] Temperature in degrees centigrade. Saturation deficiencies in millimeters of mercury.
[2] 30° minus 25°.
[3] 25° minus 20°.
[4] 20° minus 15°.

Trichogramma adults are extremely active at 25° C. after they have been reared to maturity at 15°. The physiological explanation of this excessive activity is not apparent, but it may partly account for the short lives of the adults.

Apparently very few attempts have been made to distinguish between the effects of temperature upon the development of the sexual functions or products and upon the immediate expression of those functions or products, i. e., the ultimate sexual effects of temperature acting upon the immature stages or upon the adults. Eidmann (*7*) found that when lepidopterous pupae were placed at a low temperature (unstated), the emerged adults produced fewer eggs; and Pospelov (cited by Uvarov (*30*)) states that in most species of Lepidoptera, high temperatures do not affect the development of the ovaries. Bliss (*5, p. 852*) correlated egg production in several species of leafhoppers with the temperature the day previous to oviposition and concluded that "temperature was found to condition oviposition more by its indirect effect upon egg development than by its direct action on egg deposition." Alpatov (*2*) found that when *Drosophila* is reared at 30° C., it produces fewer eggs at 25° than does an insect reared at 19°. Data presented in table 9 indicate that the opposite effect is produced on *Trichogramma*.

By reference to table 10 it will be noted that the progeny of parasites developing at 15° C. are preponderantly male, i. e., developed from unfertilized eggs. This low sex ratio must be due to a reduced potency of the male parents reared at 15°, since the number of male progeny is consistently higher than the number of female progeny throughout the lives of the parent females, thus indicating that the females have not been properly impregnated.

TABLE 10.—*Sex ratios of the progeny of pairs reared through their immature stages at different temperatures*

Temperature ° C.	♀/total	Total counted
30	0. 62	933
25	. 66	1, 788
20	. 57	614
15	. 42	2, 425

Peterson (*22*) and Schread and Garman (*25*) note that when parasitized *Sitrotroga* eggs are kept under certain conditions of refrigeration, a change in the sex ratio of the emerging parasites occurs. This is undoubtedly a matter of selective mortality between the sexes as was pointed out by Peterson (*22*), and not the result of an actual alteration of the germ plasm. The data in table 10, however, indicate that a real reduction in the potency of the males is effected if they are reared to maturity at low temperatures.

The longevity and productivity of parasites as affected by temperature—acting either upon the immature stages or upon the adults—are summarized graphically in figure 2.

It appears from tables 9 and 10 and from figure 2 that adults to be liberated in the field in a pest-control project should not be those emerging from host eggs which, for the purpose of retarding development, have been kept at a temperature lower than 20° C., for if

such adults are used, the lower sex ratio of their progeny, their markedly shorter life, and their reduced productivity, will reduce the probability of success. It would seem that artificial rearing at about 25° to 30° is conducive to a longer life and a greater number of eggs in the field, the greater longevity at 30° being about offset by the greater productivity at 25°. In building up populations in the laboratory, however, 30° C. is probably a more efficient rearing temperature than 25°,

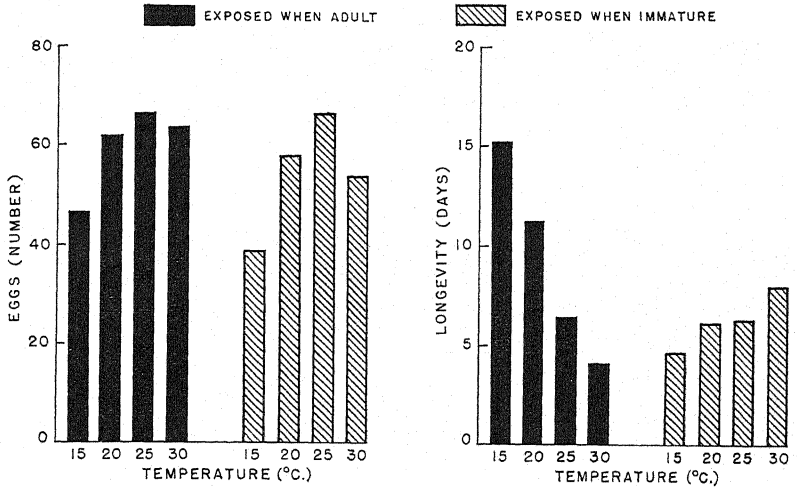


FIGURE 2.—Effects of temperature upon the productivity and female longevity of *Trichogramma*.

since the reduction in development time at 30° (about one-third less than at 25°) certainly more than offsets the advantages of higher productivity at 25°.

EFFECTS OF MOISTURE CONDITIONS

Quantitative data on the effects of humidity upon the longevity and productivity of insects are rare and, so far as the writer is aware, no attempts have been made to differentiate between the effect of moisture upon the development of the sexual capabilities and upon the process of oviposition itself. Certainly nothing of this sort has been done for *Trichogramma*.

EFFECTS OF HUMIDITY ACTING UPON THE ADULTS

In these experiments, the parasites were reared to maturity at the standard condition of 25° C. and 5-mm saturation deficiency, and immediately after emergence were fed, paired, and placed at atmospheric conditions of 0-mm (saturation), 5-mm, 10-mm and 15-mm saturation deficiency. In table 11 the longevity and productivity at these successively drier conditions are compared.

It will be noticed that at a saturation deficiency of 10 and 15 mm the longevity of both males and females is markedly reduced. Since the products of the saturation deficiencies (governing rate of evaporation) and the longevities (duration of evaporation) at 10 and 15 mm are about equal (43.0 and 45.0 respectively for the females and 44.0 and 36.0 for the males) the total amounts of water evaporated from the

parasites at these two conditions were about equal. This would indicate that in these cases the limiting lethal factor is probably desiccation and that the desiccation limit is around 40 mm—days.

TABLE 11.—*Productivity (black host eggs) and longevity of pairs spending their adult lives at different moisture conditions*

Treatment and factors observed [1]	*N*	Mean±S. E.	Difference±S. E.	Difference / S. E. difference
0 mm (saturation):				
Progeny, number	46	64. 0±1. 7		
♀ longevity, days	46	6. 8± . 3		
♂ longevity, do	44	5. 2± . 2		
5 mm:				
Progeny, number	70	66. 1±2. 5	[2] −2. 1±3. 0	0. 7
♀ longevity, days	71	6. 3± . 3	[2] . 5± . 4	1. 2
♂ longevity, do	63	5. 7± . 2	[2] −. 5± . 3	1. 7
10 mm:				
Progeny, number	57	58. 4±2. 0	[3] 7. 7±3. 2	2. 4
♀ longevity, days	57	4. 3± . 3	[3] 2. 0± . 4	5. 0
♂ longevity, do	58	4. 4± . 1	[3] 1. 3± . 2	6. 5
15 mm:				
Progeny, number	58	51. 9±1. 2	[4] 6. 5±2. 3	2. 8
♀ longevity, days	58	3. 1± . 2	[4] 1. 2± . 4	3. 0
♂ longevity, do	58	2. 4± . 1	[4] 2. 0± . 1	20. 0

[1] Temperature 25° C.; saturation deficiencies in millimeters of mercury.
[2] 0 mm minus 5 mm.
[3] 5 mm minus 10 mm.
[4] 10 mm minus 15 mm.

Since death in a saturated atmosphere must be due to other causes than desiccation, and since the longevity is about equal at saturation and at 5-mm saturation deficiency, death in the latter case also must be due to other causes than desiccation. In other words, the adult parasites are able to live out their normal span of life at about 5-mm saturation deficiency or lower, but at 10-mm or higher the longevity is markedly reduced as a result of the drying effect of the atmosphere. It will be noticed, though, that the effects upon productivity are much less pronounced than the effects upon longevity. This is to be expected since, due to the characteristically high initial egg production by *Trichogramma*, the detrimental effects of high water loss should not be reflected in the reduced oviposition of the female until most of her eggs have already been laid.

The general nature of the egg-laying curve is about the same at the various moisture conditions as at the standard condition (see fig. 1), a more rapid termination as a result of a shorter life at 10- and 15-mm saturation deficiency being the only apparent difference.

The sex ratio of the progeny is reduced as the humidity is lowered (table 12). This reduction is apparently due to the reduced initial copulation activity of the males at low humidities, since after the first laying period the sex ratio abruptly returns to normal.

TABLE 12.—*Sex ratios of progeny of pairs kept at various humidity conditions*

Saturation deficiency (mm Hg)	Females total	Total counted
0	0. 57	2, 310
5	. 66	1, 788
10	. 44	2, 476
15	. 39	2, 200

The data in tables 11 and 12 on adults placed at four different humidity conditions, suggest that field liberations can be made to best advantage when the saturation deficiency is 5 mm or less, i. e., when the relative humidity is above 84.3 percent at 30° C., 78.8 percent at 25°, or 71.6 percent at 20°.

In building up laboratory stocks, where longevity is not of much importance, since host eggs are usually presented to the parasites shortly after their emergence, any saturation deficiency less than about 10 mm, i. e., relative humidities above 68.6 percent at 30° C., 57.0 percent at 25°, or 43.0 percent at 20°, should be satisfactory.

Effects of Humidity Acting Upon the Immature Stages

In these experiments, the parasites were reared to maturity at 25° C. in atmospheres of 0-mm (saturation), 5-mm, and 15-mm saturation deficiency. Within 12 hours after emergence all adult parasites were fed, paired, and placed at the standard condition where they remained for the rest of their lives. Thus the influence of high and low humidities upon the development of the sexual structures and products was tested at the single condition. The results of these experiments are presented in table 13.

Table 13.—*Productivity (black host eggs) and longevity at 5-mm saturation deficiency of pairs reared to maturity at 0-mm, at 5-mm, and at 15-mm saturation deficiency*

Treatment and factors observed [1]	N	Mean±S. E.	Difference± S. E.	Difference / S. E. difference
0 mm (saturation):				
Progeny.....number..	63	59. 1±2. 5	.....	.....
♀ longevity.....days..	63	6. 0± .3	.....	.....
♂ longevity.....do....	65	5. 7± .1	.....	.....
5 mm:				
Progeny.....number..	70	66. 1±2. 5	[2] −7. 0±3. 5	2. 0
♀ longevity.....days..	71	6. 3± .3	[2] −. 3± .4	.7
♂ longevity.....do....	63	5. 7± .2	[2] 0± .2	.0
15 mm:				
Progeny.....number..	43	40. 3±2. 0	[3] 25. 8±3. 2	8. 1
♀ longevity.....days..	39	5. 7± .3	[3] 6± .4	1. 5
♂ longevity.....do....	45	5. 5± .3	[3] 2± .4	.5

[1] Temperature 25° C.; saturation deficiencies in millimeters of mercury.
[2] 0 mm minus 5 mm.
[3] 5 mm minus 15 mm.

It will be seen that the humidity in which the parasites are reared to maturity has no effect upon the longevity of the subsequent adults, but both higher and lower humidities (saturation and 15-mm saturation deficiency) reduce the productivity.

The sex ratios of the progeny produced are not significantly affected by the moisture conditions at which the parents are reared to adulthood. At saturation the ratio of females to the total number of progeny counted was 0.55 (2,930 counted), whereas at 15-mm saturation deficiency the ratio was 0.63 (1,266 counted).

In figure 3 the data on the influence of atmospheric moisture upon the longevity and productivity of *Trichogramma* are summarized graphically.

It will be seen that humidity has the same general influence upon egg production when it acts upon the developmental stages as when it acts upon the adults only. The effects are much more pronounced, how-

ever, when the influence is exerted on the immature stages. In other words, the effects of adverse moisture conditions are more pronounced when they act during the relatively long developmental period (about 10 days) than when they influence directly the function of oviposition during the short adult life, only the very first part of which is of much significance in the total productivity.

On the other hand, the longevity of adult parasites exposed as adults is much more markedly affected by atmospheric humidity than is the

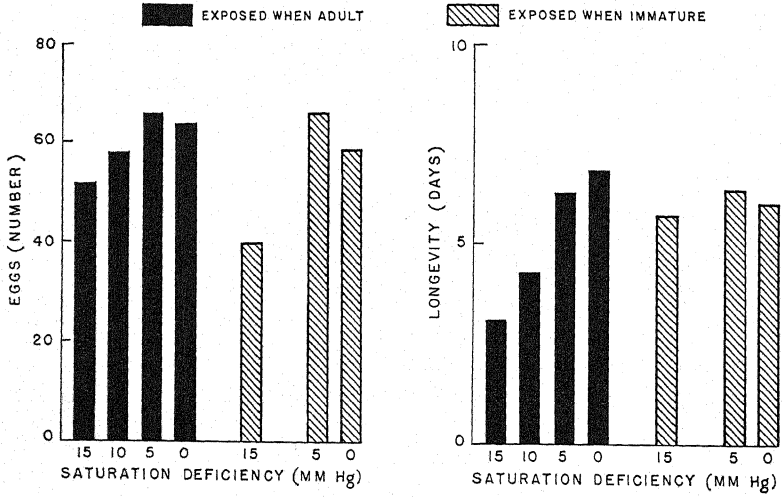


FIGURE 3.—Effects of humidity upon productivity and female longevity of *Trichogramma*.

longevity of adults exposed as eggs, larvae, and pupae. Apparently the influence of moisture during development is exerted more or less specifically upon the reproductive function and not on the general factors that determine length of life.

These data suggest that in building up both laboratory stocks and stocks for field liberation, the ideal atmospheric humidity in which to rear the parasites to maturity is within 5 mm of saturation.

SUMMARY AND CONCLUSIONS

The longevity and productivity of *Trichogramma evanescens* Westwood, reared in *Sitotroga* eggs, were tested under various conditions of feeding, withholding host eggs, pairing, superparasitism, light, temperature, and humidity. From the experimental data, the following results were obtained and conclusions drawn:

(1) The average number of progeny produced at 25° C. and 5-mm saturation deficiency is 66.1 ± 2.5, the female longevity 6.3 ± 0.3 days, the male longevity 5.7 ± 0.2 days, and the sex ratio 0.66 female.

(2) A correlation of $+0.639 \pm 0.071$ exists between the longevity of mated females and their productivity.

(3) Since females fed on dilute honey live longer and produce more progeny than unfed females, parasites should be fed before liberation in the field.

(4) Females live longer when host eggs are present than when they are absent, but if the eggs are withheld for 48 or 72 hours, female longevity is unaffected while productivity is reduced. Therefore only young parasites should be liberated in the field.

(5) Pairing reduces productivity but leaves longevity unaffected.

(6) Females emerging from superparasitized host eggs produce fewer progeny than normal females but live as long. Males reared under the same conditions, while sexually potent, are shorter-lived than normal males.

(7) Females kept in diffused light live slightly longer, than females kept in total darkness, but produce the same number of progeny.

(8) Adult longevity varies inversely as the temperature to which the adults are subjected, but directly as the temperature to which their developmental stages are exposed; the optimum temperature for productivity is about 25° C. in either case. These facts suggest that the most favorable field temperature at which to liberate the parasites for pest control is probably just under 25°, whereas the most favorable laboratory breeding temperature is about 30°.

(9) An abnormally high proportion of male progeny is produced by parasites developing at 15° C. (only 0.42 female).

(10) When adults are subjected to either low (10-mm or 15-mm saturation deficiency) or high (saturation) humidities, their productivity is somewhat reduced, but relatively less so than their longevity, and the total water lost before death is about the same at both "dry" conditions; but, conversely, when the immature stages are exposed to low or high humidities, the productivity of the subsequently emerging adults is decidedly reduced while the longevity is unaffected. The most favorable field humidity at which to liberate the parasites would appear to be 5-mm saturation deficiency or less, but laboratory breeding conditions of 10-mm saturation deficiency or less are probably satisfactory.

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MEIOTIC INSTABILITY AS AN INHERITED CHARACTER IN VARIETIES OF TRITICUM AESTIVUM [1]

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INTRODUCTION

In previous studies of varieties of *Triticum aestivum* L., Powers (*16, 17, 18*)[2] found that the cytological aberrations of nonorientation of the chromosomes in meiosis, the occcurence of univalents at metaphase, and chromatin loss in the parent plants were associated with the coefficients of variability of their progeny for weight of seed per plant, height of plant, and percentage of fruitfulness. These results indicated that in some cases meiotic instability is an important character in a breeding program. For this reason it seemed desirable to study the relative importance of heredity and environment in the determination and differentiation of this character.

MATERIALS AND METHODS

The frequency of occurrence of chromatin loss was employed as a measure of meiotic instability. The reasons for using this character were twofold. (1) Chromatin loss was found to be highly correlated with nonorientation and the occurrence of univalents (*17, 18*). Thus of the single determinations, it seemed best adapted as a criterion of meiotic instability. (2) It permitted the collection of data on a larger number of plants than would have been possible if a number of meiotic irregularities had been studied. To determine chromatin loss, 500 immature microspores, still in the form of quartets, were examined for each plant and the number showing micronuclei was recorded.

Five varieties of *Tritucum aestivum*, namely, Marquis, Supreme, Thatcher, Double Cross 2305, and H–44, were selected for use in this study. Marquis and Thatcher were included because in previous investigations (*17, 18*) both of these varieties were found to be comparatively free from meiotic instability. In preliminary studies, Double Cross 2305 and H–44 had shown a fairly high degree of meiotic instability while Supreme was known to be variable in many agronomic characters and it seemed, therefore, that this variety might also be meiotically unstable.

Plants of the five varieties were studied cytologically in 1931. In 1932, 1933, and 1936, progeny rows were grown from various selections and in all cases from seed produced under bag to insure self-pollination. Plants having a low percentage of micronuclei were selected in 1931 from Thatcher, Marquis, Double Cross 2305, and H–44; plants having a high percentage of micronuclei were selected from Double Cross

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[2] Reference is made by number (italic) to Literature Cited, p. 451.

2305 and H–44; and a plant intermediate between low and high was selected from Marquis. The individual plant selections in 1931 and their progeny in later generations will be designated as lines. The progenies of these eight lines were studied cytologically in 1932. In 1933 each line was the progeny of a randomly selected individual plant of 1932 and, in 1936, individual progenies of from 7 to 10 plants of each line were studied. Thus conclusions drawn from the study on the inheritance of meiotic instability are based on 3 years of progeny testing.

The histories of the different varieties are of value in interpreting the results and therefore are given here. Marquis resulted from a cross of two aestivum varieties and Supreme was produced by selection from Red Bobs, an aestivum variety. Thatcher originated from a cross of a selection of Marquis × Iumillo (a variety of *Triticum durum*) with a selection from Kanred × Marquis, (*13*) and Double Cross 2305 was a sister selection of Thatcher. H–44 was produced by McFadden (*15*) from a cross between Yaroslav emmer and Marquis.

For the first 3 years of the test, the effects of the environment, if any, could not be separated from possible genetic effects. Therefore, in order to collect information regarding environmental effects, a more intensive study including different dates of collecting the cytological material was set up for 1936.

For the cytological studies, part of the material was killed and fixed in Allen's modification of Bouin's fixative and the slides were stained by Newton's iodine gentian-violet method described by LaCour (*14*). For the majority of the studies, Belling's (*9*) iron aceto-carmine smear method was used, the material having been killed and fixed in acetic alcohol (3 parts of absolute alcohol : 1 part of glacial acetic acid) and stored in 70-percent alcohol.

EXPERIMENTAL RESULTS

INHERITANCE OF MEIOTIC INSTABILITY

The data having a bearing on the inheritance of meiotic instability were evaluated under the following classifications, the percentage of micronuclei for the varieties from which the lines used in this study were selected, 3 years of progeny testing, homozygosity of lines and cultures, and the number of monosomic plants obtained from each line and variety.

The frequency distributions of percentage of micronuclei for the varieties from which the lines used in this study were established are given in table 1.

The five varieties occurred in three distinct groups on the basis of percentage of micronuclei. No plants were found in either Thatcher or Marquis with more than 3 percent of micronuclei while none of the plants of Supreme had less than 3 percent. The plants of H–44 and D. C. 2305 ranged from less than 1 percent to more than 19 percent of micronuclei. One plant of each of these two varieties and four plants of Supreme showed more than 19 percent of mironuclei. Metaphase and anaphase I studies of these plants revealed that each was monosomic, having only 41 somatic chromosomes. These results agree with those reported by Powers (*17*) for monosomic plants of *Triticum aestivum*. Since the high percentage of micronuclei of monosomic

plants is due largely to the loss of the univalent chromosome, these plants have not been included in the averages for the varieties. That differences between the varieties were statistically significant can be seen from the data given at the bottom of table 1. Likewise, the monosomic plants were not included in these calculations. If these differences are characteristic of certain varieties and strains, it is evident that they are inherited. To determine whether characteristic differences do occur, progeny tests were conducted for 3 years.

TABLE 1.—*Frequency distribution of percentage of micronuclei for the varieties from which the lines used in this study were established, and analysis of variance to determine whether the differences between varieties could be due to the errors of random sampling*

FREQUENCY DISTRIBUTION

Variety	Total plants	Plants with the indicated percentage of micronuclei					Mean percentage of micronuclei
		0–1.0	1.1–3.0	3.1–10.0	10.1–19.0	19.1–26.0	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Thatcher	25	18	7				0.8
Marquis	26	17	9				.9
H–44	20	4	6	7	2	1	4.1
D. C. 2305	11	4	1	2	3	1	5.5
Supreme	9			4	1	4	8.3

ANALYSIS OF VARIANCE

Variance	*D. F.*	Mean square	*F*	Value of *F* for a *P* of 0.01
Between varieties	4	201.436	51.21	3.56
Within varieties	80	3.934		

The data collected in the three generations of progeny testing are given in table 2. These data were analyzed to determine whether the differences could be accounted for by the errors of random sampling.

TABLE 2.—*Yearly average percentage of micronuclei for each line, number of plants making up the averages, and the analysis of variance for each year*

PERCENTAGE OF MICRONUCLEI AND NUMBER OF PLANTS

Line	Average percentage of micronuclei for—						Plants making up the yearly averages			
	1931	1932	1933	1936	1931–33	1931–36	1931	1932	1933	1936
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Number*	*Number*	*Number*
Thatcher-L	0.3	1.1	0.6	1.6	0.67	0.90	1	10	7	51
Marquis-L	.5	.5	1.3	1.4	.77	.92	1	10	10	53
Marquis-M	2.2	3.3	3.0	3.3	2.83	2.95	1	10	10	58
D. C. 2305-L	.7	.7	2.2		1.20		1	3	8	
D. C. 2305-M	15.6	.8	3.1		6.50		1	8	8	
H–44-L	.7	2.1	1.8		1.53		1	10	8	
H–44-M	13.3	3.2	3.2	3.3	6.57	5.60	1	1	10	40
H–44-H	13.0	4.8	12.3	5.2	10.03	8.82	1	10	7	42

TABLE 2.—*Yearly average percentage of micronuclei for each line, number of plants making up the averages, and the analysis of variance for each year*—Contd.

ANALYSIS OF VARIANCE

Year	*D. F.*		Mean square		*F*	Value of *F* for a P of 0.01 [1]
	Between lines	Within lines	Between lines	Within lines		
1931 [2]	4	80	201.4358	3.9336	51.21	3.56
1932	6	54	42.1162	3.3995	12.39	3.19
1933	7	60	98.1947	5.3638	18.31	2.97
1936	4	247	115.3965	6.4470	17.90	3.41

[1] The values of *F* for *P* of 0.01 are for the nearest degrees of freedom given by Snedecor (*20*).
[2] For 1931 the analysis given is for "between varieties" and "within varieties."

Since several progenies were grown for each line in 1936 under randomization, the mean square within lines seems reliable as an error in obtaining the *F* value. It was used also in the other years. For every generation as represented by years, the *F* value was considerably larger than that necessary to give a *P* of 0.01. It may be concluded that for every generation some of the differences between lines were too great to be attributed to the errors of random sampling and therefore some of the differences may be judged statistically significant.

To determine which differences between the lines selected for high and low meiotic instability may be judged statistically significant, the standard error of the difference was used. The differences between the high and low selections divided by the standard error of their differences are given in table 3.

TABLE 3.—*Differences between lines divided by their standard error*

Lines compared	Difference divided by the standard error		
	1932	1933	1936
Marquis-L with Marquis-M	−3.40	−1.60	−4.00
Double Cross 2305-M with D. C. 2305-H	−.08	−.80	
H–44–L with H–44–M	−.60	−1.30	
H–44–M with H–44–H	−.80	−7.80	−3.40
H–44–L with H–44–H	−3.30	−8.80	

The high values for the difference divided by the standard error leaves little room for questioning that the lines within Marquis differ significantly and that H–44–M and H–44–L are significantly different from H–44–H.

The most critical evidence on the inheritance of meiotic instability is furnished by the sign (plus or minus) of the difference. In table 3 the minus signs indicate that the differences were all negative. The negative values indicate that the lines selected for low meiotic instability were uniformly lower in percentage of micronuclei than the lines from parents of higher variability. Thus, these data are rather conclusive in showing that meiotic instability is inherited.

The material of the five lines studied in 1936 consisted of the progenies of 42 plants. The percentage of micronuclei of 38 of these 42

plants was determined in 1933. The correlation coefficient for percentage of micronuclei of the 38 plants with the average percentage of micronuclei of their progenies in 1936 was 0.77, a highly significant value. This lends further support to the conclusion that heritable differences in meiotic instability existed.

In 1936 the experiment was designed to yield some information regarding the nature of the inheritance of meiotic instability. In this regard, it was of interest to know whether homozygous lines, significantly different in percentage of micronuclei, had been obtained. The data having a bearing on this problem are given in table 4.

TABLE 4.—*Homozygosity of lines in 1936 for percentage of micronuclei as determined by the analysis of variance*

Lines	*D. F.* for—		Mean square for—		*F*	Value of *F* for a *P* of [1]—	
	Cultures	Error	Cultures	Error		0.05	0.01
Thatcher	8	[2] 37	3.449	3.246	1.06	2.22	3.07
Marquis–L	8	[3] 39	5.250	2.555	2.05	2.18	2.99
Marquis–M	9	[4] 43	5.882	1.686	3.49	2.18	2.99
H–44–M	6	[4] 28	.066	2.338			
H–44–H	6	30	62.073	11.286	5.50	2.42	3.47

[1] The values of *F* for *P* values of 0.05 and 0.01 are those values for the nearest degrees of freedom given by Snedecor (*20*).
[2] 3 values missing and interpolated.
[3] 1 value missing and interpolated.
[4] 2 values missing and interpolated.

Microsporocytes were collected from one plant of each culture on each of 6 days from June 22 to June 27, inclusive. The material was not at the proper stage for determining the percentage of micronuclei in two plants each of Marquis–M and H–44–M and three plants of Thatcher. In addition, one plant of Marquis–L was monosomic and therefore was omitted from the calculation. In calculating the analysis of variance, values for these plants were interpolated, using the formula given by Yates (*21*).

From the *F* values in table 4, it would seem that Thatcher and H–44–M are homozygous for percentage of micronuclei. The *F* value for Marquis–L almost reaches that expected for a *P* of 0.05 and the *F* value for Marquis–M exceeds the value of F for a *P* of 0.01. These results indicate that the last two lines are not homozygous for percentage of micronuclei. However, such a conclusion is difficult to justify from an examination of table 2. As shown by the mean percentage of micronuclei for years, the progeny test for Marquis–L would lead one to suspect that this line was as stable as Thatcher and therefore homozygous. Likewise, uniformity of the mean percentage of micronuclei for Marquis–M in different years was striking and certainly would lead one to believe that this line was homozygous for the character in question.

Since the data indicate that Thatcher and H–44–M are homozygous, it is desirable to know whether the difference between these two lines can be accounted for by the errors of random sampling. From table 5 it can be seen that the odds against the differences between the means of these two lines being due to the errors of random sampling are very large.

TABLE 5.—*Comparison of the mean percentage of micronuclei of the 5 lines studied in 1936 by means of the standard error of the difference*

Comparison	Difference	D/S. E.	Comparison	Difference	D/S. E.
Thatcher with Marquis-L	0.2	0.4	Marquis-L with H-44-M	1.9	3.6
Thatcher with Marquis-M	1.7	3.6	Marquis-L with H-44-H	3.8	7.3
Thatcher with H-44-M	1.7	3.2	Marquis-M with H-44-M	.0	.0
Thatcher with H-44-H	3.6	6.9	Marquis-M with H-44-H	1.9	3.7
Marquis-L with Marquis-M	1.9	4.0	H-44-M with H-44-H	1.9	3.4

The variation of plants of H–44–H in percentage of micronuclei in 1933 indicated that this line was not breeding true for this character. This was substantiated by the behavior of the progenies of these plants in 1936 (table 4).

To determine whether any of the plants of H–44–H studied in 1933 were homozygous for factors determining meiotic instability, a more extensive study of their progenies in 1936 was made. Material was collected from all of the available plants of the seven cultures of H–44–H and data on percentage of micronuclei were obtained for a total of 129 plants. These data are summarized in table 6.

TABLE 6.—*Number of plants, frequency distribution of plants in each line for percentage of micronuclei, and average percentage of micronuclei for cultures of H–44–H in 1936*

Culture No.	Plants studied	Plants showing indicated percentage of micronuclei					Average percentage of micronuclei
		0–1.0	1.1–3.0	3.1–10.0	10.1–19.0	19.1–26.0	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
C38	17	1	5	8	3	----	6.9
C9	20	3	4	7	4	2	6.1
C19	18	1	6	6	3	2	6.0
C5	21	2	9	6	4	----	4.7
C18	21	5	5	8	3	----	4.5
C22	13	4	5	4	----	----	2.8
C4	19	5	11	1	----	2	1.7

Six of the 129 plants had more than 19 percent of micronuclei. The chromosome number of three of these plants, namely, the two occurring in culture No. 4 and one in culture No. 19, was determined and each plant was found to be monosomic. It seems probable that the other three plants may also have been monosomic since the percentage of micronuclei shown by each is within the range for monosomic plants. For this reason, these six plants were not included in the calculation of the means for the cultures.

Five of the seven cultures of H–44–H had plants with less than 1 and more than 10 percent of micronuclei while, in the remaining two cultures, all plants with 42 chromosomes showed less than 10 percent of micronuclei. This range in percentage of micronuclei of the different plants indicated that the seven cultures were still segregating for meiotic instability. Since the 500 microspores of each plant were counted in groups of 100, it was possible to measure the variation within plants and determine whether significant differences between plants existed. For this purpose an analysis of variance was calculated for each culture (table 7).

TABLE 7.—*Summary of the analysis of variance of percentage of micronuclei for each culture of H–44–H grown in 1936*

Culture No.	D. F.		Mean square		F	Value of F for P of—[1]	
	Between plants	Within plants	Between plants	Within plants		0.05	0.01
C38	16	68	147.1280	10.7058	13.74	1.89	2.45
C9	17	72	161.5350	8.5833	18.82	1.89	2.45
C19	15	64	145.8133	9.2312	15.80	1.89	2.45
C5	20	84	91.0295	7.0520	12.91	1.65	2.03
C18	20	84	105.2314	7.9000	13.32	1.65	2.03
C22	12	52	31.3718	4.1077	7.64	1.95	2.56
C4	16	68	5.1941	2.4882	2.09	1.89	2.45

[1] Values for nearest degrees of freedom given by Snedecor (*20*).

For six of the cultures the value of F for comparing mean square between plants with mean square within plants was greater than F for a P of 0.01. In culture 4, the value of F gave P between 0.05 and 0.01. Therefore, it appears that none of these lines was homozygous for meiotic instability.

The number of monosomic plants obtained from each line and the total number from each variety together with the mean percentage of micronuclei are shown in table 8.

TABLE 8.—*The number of monosomic plants obtained from each line and variety*

Line or variety	Total plants	Monosomic plants	Mean percentage of micronuclei	Line or variety	Total plants	Monosomic plants	Mean percentage of micronuclei
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
Thatcher	93	0	0.90	H–44–L	18	0	1.53
Marquis–L	73	1	.92	H–44–M	51	0	5.60
Marquis–M	78	0	2.95	H–44–H	129	[1] 6	8.82
Total [2]	177	1		Total [2]	218	7	
D. C. 2305–L	11	0	1.20	Supreme	9	4	8.30
D. C. 2305–M	16	0	6.50				
Total [2]	38	1					

[1] 3 of these plants were determined to be monosomic on the basis of the percentage of micronuclei.
[2] Total for the variety includes data for 1931 before the lines within the varieties were established.

It is evident that in this investigation the varieties showing the highest percentage of micronuclei tended to have the greater number of monosomic plants. Since monosomic plants arise from chromatin loss such an association would be expected. Thus, lines exhibiting a high degree of meiotic instability are rendered even more unstable by the occurrence of these monosomic plants. An occasional monosomic plant among the progeny of such meiotically stable varieties as Thatcher and Marquis would be expected. One such plant was found in Marquis.

THE EFFECT OF THE ENVIRONMENT ON MEIOTIC INSTABILITY

The data on the effect of environment upon meiotic instability are given in table 9.

TABLE 9.—*The average percentage of micronuclei of each line for each of the 6 days of collection in 1936*

Line or variety	Average percentage of micronuclei for—					
	June 22	June 23	June 24	June 25	June 26	June 27
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Thatcher	0.8	0.9	0.6	2.4	2.1	2.5
Marquis-L	.5	.9	1.1	1.8	1.8	1.8
Marquis-M	2.8	3.0	2.8	3.7	4.2	3.8
H-44-M	2.5	1.4	1.8	4.1	3.1	6.9
H-44-H	2.1	4.9	3.5	6.3	8.7	5.7
Average	1.7	2.2	2.0	3.7	4.0	4.1

Microsporocytes were collected from one plant of each culture on each of 6 days in 1936. There was a marked difference between the percentage of micronuclei for the first 3 days and the last 3 days of collection. For purposes of evaluation it is necessary to know whether the differences noted could be attributed to the errors of random sampling. If they are due to the errors of random sampling, the probability is 0.5 that any value of a given line for the last 3 days will exceed any value of the same line for the first 3 days. Then the probability of this same value exceeding all three values for the first 3 days is $(0.5)^3$ and likewise the probability of all three values of the last 3 days for any one line exceeding all three values for the first 3 days for the same line is $(0.5)^3\ (0.5)^3\ (0.5)^3$. Completing these calculations it is seen that the odds are 499:1 against such a result occurring due to the errors of random sampling. Since the values for the last 3 days of collection of each line exceeded the value of the first 3 days of collection for the same line, it is evident that the material collected the first 3 days is significantly lower in percentage of micronuclei than that collected the last 3 days of the investigation. From this it may be concluded that environmental conditions prior to the collection of the material are a factor in determining the percentage of micronuclei that a given plant possesses.

DISCUSSION

One of the major advances in the development of superior varieties of aestivum wheat was the transfer of the field resistance to black stem rust of varieties of the emmer and durum group to bread wheat varieties. Marquillo, developed at the Minnesota Agricultural Experiment Station from a cross of Iumillo durum with Marquis, and Hope and H–44, developed from a cross of Yaroslav emmer × Marquis made by McFadden (*15*), are notable examples of aestivum varieties which have received genes for rust resistance from varieties of the emmer and durum group. Two of these varieties, Marquillo and H-44, have been investigated cytologically and found to be significantly more irregular in meiosis than the standard variety, Marquis (Powers (*16, 17, 18*) and the present investigations). Although it cannot be concluded from these two cases that varieties produced by interspecific hybridization will all show meiotic instability, selections produced in this manner should be investigated cytologically because of the possibility that they might exhibit such variability.

The results obtained with Supreme indicate that meiotic irregularity is not confined to selections from interspecific hybrids. As stated previously, Supreme was produced by selection from Red Bobs, an aestivum variety. Yet Supreme was found to be even more unstable in 1931 than H–44. The possibility that Supreme may have arisen as a result of natural crossing must of course not be overlooked.

The cytological behavior of Thatcher and D. C. 2305 is of considerable interest. These varieties are sister selections from the double cross (Iumillo × Marquis) × (Kanred × Marquis) and both show the same type of rust resistance as their (Iumillo × Marqius) parent. Thatcher was at least as stable cytologically as Marquis while D. C. 2305 showed a significantly higher degree of meiotic instability. These results indicate that although selections obtained from hybrids between species may be unstable, cytologically stable varieties having the desirable characteristics obtained from the related species may be produced by crossing with a variety which does not show meiotic irregularities.

The investigations reported in this paper show that differences in meiotic instability are conditioned to a considerable extent at least by heritable factors. The variation between plants of the same variety suggested the possibility of selection of stable lines even from such varieties as H–44. The different lines obtained from Marquis and H–44 indicate that selection has been effective in isolating types that differ significantly in cytological behavior. In these investigations, selection was practiced only in 1931. It is possible that by continued selection of plants showing a low percentage of micronuclei, even more stable lines than those obtained in this study might be isolated. This possibility should be investigated.

The design of the experiment in 1936 was such that some information could be derived from the data obtained regarding the nature of the heritable differences conditioning differences in meiotic instability. The meiotic irregularities might arise from genetic factors which govern chromosome behavior, from structural differences between synapsing chromosomes or perhaps from similarities between chromosomes of different genomes which would lead occasionally to the formation of trivalents or quadrivalents at metaphase I. If the occurrence of micronuclei is to be accounted for by structural differences between synapsing chromosomes, inbreeding should tend to eliminate these differences and all homozygous lines should be fluctuating about the same mean percentage of micronuclei. Actually, apparently homozygous lines, differing significantly in percentage of chromatin loss, were obtained. The mean percentages of micronuclei of Thatcher, Marquis–L, Marquis–M, and H–44–M remained relatively constant during the 3 years of progeny testing whereas the elimination of structural differences due to inbreeding should cause a decrease in chromatin loss.

That the differences in meiotic instability could be accounted for by similarity between chromosomes of different genomes likewise seems improbable. The occurrence of occasional multivalent associations, reported by Powers (*17*) and other workers, indicates that partial homology does exist between chromosomes of different genomes. Such partial homology, in the absence of structural differences between

members of the two respective homologous pairs, should lead to the formation of quadrivalents instead of trivalents and univalents in a majority of cases. The writers do not know of any evidence that quadrivalents lead to chromatin loss. On the contrary, the senior writer (unpublished data) has investigated the occurrence of chromatin loss in *Arrhenatherum elatius* (L.) Mert. and Koch and a tetraploid strain of *Agropyron cristatum* (L.) Beauv., both of which behave cytologically like autotetraploids and in both of which seven quadrivalents per microsporocyte occasionally occur. In five plants of *A. elatius*, the percentage of micronuclei varied from 1.0 to 6.8 while in six plants of *A. cristatum* the percentage of micronuclei varied from 0.0 to 3.0 In *Triticum aestivum*, Powers (*17*) found that the correlation between the occurrence of multivalents and percentage of micronuclei was not statistically significant and that the frequency of trivalents was not great enough to account for the number of univalents found at metaphase I. In addition, Powers (*17*) found that nonorientation of bivalents at metaphase I was highly correlated with the occurrence of micronuclei and it is difficult to account for failure of bivalents to orient on the equatorial plate either on the basis of structural differences between synapsing chromosomes or partial homology of chromosomes of different genomes. Thus, the results indicate that genetic factors were involved in determining differences in percentage of chromatin loss in this study. Genetic factors affecting meiosis have been reported in maize by Beadle (*1, 2, 3, 4, 5, 6, 7*) and Beadle and McClintock (*8*), in *Datura* by Bergner, Cartledge, and Blakeslee (*10*), and in wheat by Sapehin (*19*). In addition, failure of pairing which may have been conditioned by genetic factors has been reported in *Nicotiana tabacum* L. by Clausen (*11*). H–44–H was still segregating in 1933. This line was investigated more extensively in 1936 and the seven cultures were all found to be segregating for percentage of micronuclei. Culture 4, which had an average of 1.7 percent of micronuclei for 19 plants and culture 22 with a mean of 2.8 percent of micronuclei for 13 plants had a considerably lower variance between plants than the remaining five cultures. In these latter cultures, the range in percent of micronuclei between plants was as great as the range of H–44 in 1931. Thus, after 3 successive years of individual plant selection, no plants were obtained in this line which appeared to be approaching homozygosity for high meiotic irregularity. Although the data were not extensive, the failure to obtain plants homozygous for high meiotic instability suggests that structural differences of the chromosomes in addition to genetic and environmental factors may have been involved in conditioning the high percentage of chromatin loss in this line.

The effect of environment on meiotic irregularity, shown by differences between dates of collection of the cytological material, indicates that comparisons between plants collected at different dates or under different environmental conditions must be interpreted with caution. Since environmental as well as heritable factors are involved in conditioning differences in meiotic instability, experiments designed to control and properly evaluate the experimental error are as necessary in studying this character as any other quantitative character.

SUMMARY

The occurrence of chromatin loss, measured by the percentage of immature microspores showing micronuclei, was studied in five varieties of *Triticum aestivum* L., namely Marquis, Thatcher, D. C. 2305, H–44, and Supreme.

In Marquis and Thatcher all plants had less than 3 percent of micronuclei, in D. C. 2305 and H–44 plants with less than 1 percent and more than 19 percent of micronuclei were found, and all plants of Supreme had more than 3 percent of micronuclei in 1931. The relation of these data to the origin of the varieties is discussed.

Results of 3 years of progeny tests indicated clearly that heritable differences in meiotic instability occurred.

A correlation coefficient of 0.77 between the percentage of micronuclei of 38 plants in 1933 and the average percentage of micronuclei of their progenies in 1936 was obtained.

Lines differing significantly in percentage of micronuclei were isolated by selection both in Marquis and H–44. Thatcher, Marquis–L, and H–44–M were apparently homozygous in 1936 and had 1.6, 1.4, and 3.3 percent of micronuclei, respectively. H–44–H had 5.2 percent of micronuclei in 1936 and was still segregating.

The isolation of homozygous lines, significantly different in percentage of micronuclei, indicated that genetic factors were involved in conditioning meiotic instability. The failure to isolate lines homozygous for high meiotic instability in H–44–H suggested that structural differences of the synapsing chromosomes may also have been involved.

Significant differences between dates of collection of microsporocytes in 1936 indicated that the environment may also be a factor in determining differences in meiotic irregularities.

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QUALITY STUDIES IN THE WHEAT-BREEDING PROGRAM AT THE MINNESOTA AGRICULTURAL EXPERIMENT STATION [1]

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INTRODUCTION

In breeding for improved spring and winter wheats in Minnesota, milling and baking tests were used as an aid in selecting strains of satisfactory quality.

Rather wide crosses have been used in spring wheat breeding to obtain resistance to stem rust. This resistance was obtained originally from 14-chromosome wheats, which were of poor quality for bread-making purposes. The data collected, therefore, seem satisfactory for measuring the value of milling and baking trials in the selection of wheats for quality among diverse types.

The major purpose of the winter wheat breeding was to obtain varieties as winter hardy as Minturki but more satisfactory in milling and baking quality. Crosses between spring and winter wheats have been used for this purpose and also to obtain varieties of winter wheat resistant to stem rust. In some respects the winter wheats also were very diverse in milling and baking qualities.

When the new strains are grown first in rod-row trials no information is available regarding their milling and baking qualities. In these trials, varieties with apparent low quality have been continued in the tests for at least two consecutive seasons before they have been discarded.

The strains in the $\frac{1}{40}$-acre plot trials, for the most part, have proved of rather satisfactory quality in previous trials. Standard varieties have been included along with new strains, and in the spring wheat varietal trials the rust-resistant varieties of poor baking quality, Marquillo and Hope, have been included.

The data available seemed rather satisfactory for determining the value of milling and baking trials in the breeding program.

PREVIOUS STUDIES OF THE INHERITANCE OF QUALITY

Examples of the early application of milling and baking tests to the testing of new varieties may be found in the reports of Hays and Boss (*7*),[2] Guthrie (*4*), and Harcourt (*5*). From a study of these earlier reports, as well as from their own breeding experiments, Humphries and Biffen (*8*) concluded that baking "strength" and "weakness"

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[2] Reference is made by number (italic) to Literature Cited, p. 464.

probably constituted a simple Mendelian pair of characters. Saunders (*11*) disagreed with this conclusion and favored the view that there was not simple inheritance of baking qualities. Biffen (*1*) presented further data to support his conclusion but later (*2*) agreed with Saunders (*12*) that strength was complex in inheritance.

After it had become generally realized that baking strength is not inherited as a unit character but is an extremely complex property, conditioned by both inherited and environmental factors, the methods for determining quality characteristics underwent a long period of development, finally evolving into the present system in which baking quality is divided into a large number of factors, such as protein content, wet-gluten content and characteristics, diastatic activity, loaf volume, grain and texture of crumb, color of crumb and of crust, smoothness of crust and shape of loaf, and many others.

Zinn (*15*) first attempted the application of statistical analysis to the individual factors comprising the baking strength of varieties of wheat grown under comparable conditions, with the result that he found crude protein content to be an inherent varietal characteristic, since he obtained an interannual correlation coefficient of 0.38 in a study of 40 pure lines of wheat. He also found a direct relationship between protein content and loaf volume within pure lines of a single variety.

Hayes et al. (*6*) report a general tendency for positive interannual correlations between the results obtained in different seasons when wheats of a diverse nature were grown under comparable conditions. The characters studied were protein content, loaf volume, flour percentage, and color and texture score of the cut loaf. All possible interrelationships of these characters were studied by correlation methods, without establishing many definite and consistent relationships. Protein content showed no consistent relation to loaf volume. There was some indication of a general tendency for positive correlations between loaf volume, color score of loaf, and texture score of loaf, but these relationships were not consistent under all conditions.

Waldron and Mangels (*13*) studied the relationship between protein content, water absorption of the flour, and volume of loaf in a series of wheats that were diverse in genetic origin but grown under conditions of uniform soil and treatment. Correlation of protein and water absorption and of water absorption and loaf volume was generally small and not significant. Protein and loaf volume gave fairly high correlations, the average for the nursery tests over a 4-year period being $r=0.441$.

MATERIAL AND METHODS

The spring and winter wheats used in the milling and baking studies consisted of those strains or varieties that were being tested for disease resistance and desirable agronomic characters in the plant-breeding program of the Minnesota Agricultural Experiment Station during the years 1927–33. The varieties used in the tests were all semihard or hard red types; no soft wheats were included.

The samples used in the spring wheat rod-row tests were composites of grain from the border rows from the nursery plot trials made at the four State experiment stations located at University Farm, Waseca, Morris and Crookston. blended so as to secure comparable samples of

all varieties. Samples from the winter wheat rod-row trials were composites of grain from two stations, University Farm and Waseca. The samples obtained from the 1/40-acre plots on each of the four stations were milled separately. These samples were not milled in replicate, as the grain available was insufficient, but baking tests were always replicated. Usually the bakings were duplicated, but occasionally as many as eight replicate bakings were made. The milling method used was that described by Markley (*9*), and the official baking procedure was that of the American Association of Cereal Chemists as reported by Blish (*3*) and Werner (*14*) with certain supplements that experience had shown to be desirable. Preliminary tests showed that the flour of the 1930 and 1931 crops, of both spring and winter wheat, was deficient in diastase. The use of 3 percent of high diastatic flour, as described by Markley and Bailey (*10*), was found to correct this deficiency and hence was used for the tests made with these crops. The characters from these milling and baking tests that were subjected to correlation analysis were test weight and protein content of the wheat, yield of straight grade flour, absorption, loaf volume, crumb color, crumb texture, and crumb grain.

To show the variation between the varieties grown and tested for milling and baking qualities, data for the more important characters of three varieties of spring wheat and of two varieties of winter wheat, together with the range of variability in each character for the varieties grown in each of the years at the four stations, are given in table 1.

The data given in table 1 show that for each of the characters there was considerable variation between the years, within a given variety, as well as a fairly wide range between the varieties grown each year. The varieties subjected to these milling and baking tests appeared to have desirable agronomic characteristics and disease resistance, and the data are those which would be obtained in any wheat-breeding improvement program.

The spring wheat varieties Marquis, Ceres, and Marquillo and the winter wheats Minturki and Minhardi were grown each year in the plots and rod-row nurseries during the entire period of the study. Most varieties or strains were tested for milling and baking qualities for at least 2 years before being discarded from the trials.

TABLE 1.—*Individual data on certain varieties and range of variability of all varieties for milling and baking quality of wheat grown at 4 Minnesota stations*

SPRING WHEAT 1/40-ACRE PLOTS

Station	Year	Test weight				Total flour				Crude protein N×5.7				Loaf volume				Color				Texture			
		Marquis	Ceres	Marquillo	Range	Marquis	Ceres	Marquillo	Range	Marquis	Ceres	Marquillo	Range	Marquis	Ceres	Marquillo	Range	Marquis	Ceres	Marquillo	Range	Marquis	Ceres	Marquillo	Range
		Lb.	*Lb.*	*Lb.*	*Lb.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Cc*	*Cc*	*Cc*	*Cc*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
University Farm	1929	59	58	60	56–60	71	73	73	71–77	11	11	12	10–13	383	380	428	380–428	96	95	91	91–99	98	97	96	96–99
	1930	57	57	55	54–60	74	72	76	71–77	12	12	11	11–13	482	495	522	473–522	100	99	95	96–100	99	99	98	96–100
	1931	50	51	50	48–55	64	68	71	60–72	19	18	18	18–20	467	545	402	402–645	98	99	92	91–101	98	101	95	95–102
	1932	55	57	53	53–57	74	72	74	69–75	16	16	16	15–18	512	527	605	405–627	98	99	96	94–101	100	101	102	97–102
	1933	58	60	56	56–61	72	74	74	71–77	18	17	18	17–20	645	645	642	595–740	97	96	92	92–99	99	100	97	97–100
Waseca	1929	57	59	53	53–59	72	74	75	66–75	11	12	12	11–13	384	448	389	384–479	94	93	92	91–97	95	95	94	92–98
	1930	54	57	53	53–58	72	74	76	69–78	12	14	12	12–14	502	545	620	502–603	98	99	96	95–100	97	98	99	96–100
	1931	57	58	56	56–59	70	71	73	68–75	15	15	16	13–19	437	465	490	392–615	99	97	92	92–103	99	100	99	97–102
	1932	55	55	53	52–58	74	73	72	69–76	15	15	16	14–16	570	547	587	392–622	101	99	93	93–101	101	101	101	96–102
	1933	61	62	61	60–63	76	74	77	74–78	13	13	14	13–17	537	555	582	527–677	97	98	93	96–99	96	97	96	95–99
Morris	1929	56	56	56	56–61	73	74	76	69–78	12	12	13	11–14	413	428	463	413–489	95	94	91	91–99	97	98	96	96–99
	1930	57	57	54	53–60	73	73	76	73–80	13	14	13	11–14	502	577	567	492–618	99	98	93	93–100	99	94	99	94–100
	1931	56	57	54	54–57	72	70	73	66–81	16	16	16	16–18	455	502	485	405–555	----	----	----	----	100	100	100	99–102
	1932	54	56	52	52–58	73	73	73	70–75	16	16	17	15–17	500	577	505	462–600	100	99	93	93–100	98	101	99	96–101
	1933	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Crookston	1929	52	58	54	50–60	71	71	75	68–79	15	13	16	12–16	478	431	473	415–499	96	96	93	93–105	98	98	97	93–105
	1930	59	59	57	57–60	76	76	77	74–80	11	11	12	11–14	502	503	530	493–592	99	99	95	95–101	98	98	96	95–100
	1931	56	59	58	55–60	72	78	75	67–78	15	15	16	14–17	485	517	552	417–560	100	99	95	93–100	100	100	101	99–101
	1932	55	55	52	52–57	70	73	73	70–74	16	16	17	15–17	557	570	445	445–605	101	100	92	92–101	99	99	97	97–100
	1933	62	62	62	54–63	78	77	72	72–80	13	13	13	13–16	452	550	532	452–645	97	99	93	93–100	96	99	96	95–100

SPRING WHEAT ROD ROWS

University Farm, Waseca, Morris, and Crookston.	1929	55	56	53	52–59	75	78	77	72–80	13	13	14	13–16	423	466	463	393–505	96	99	92	91–103	97	99	94	94–101
	1930	56	57	54	53–59	72	76	75	72–79	15	14	15	14–16	570	603	566	468–730	98	98	94	92–100	97	99	96	93–101
	1931	54	55	53	53–57	72	68	71	65–77	17	17	18	16–18	503	570	420	418–670	101	100	94	93–101	100	100	98	97–101
	1932	54	56	52	52–57	72	75	73	72–77	15	16	16	15–17	562	625	512	472–597	100	102	92	92–101	101	102	100	100–102
	1933	59	60	58	57–61	76	75	77	75–78	16	16	17	13–17	640	607	650	485–582	98	100	93	93–102	99	99	97	93–100

WINTER WHEAT ROD ROWS

Station	Year	Test weight			Total flour			Crude protein			Leaf volume			Color			Texture		
		Min-turki	Min-hardi	Range	Min-turki	Min-hardi	Range	Min-turki	Min-hardi	Range	Min-turki	Min-hardi	Range	Min-turki	Min-hardi	Range	Min-turki	Min-hardi	Range
		Lb.	*Lb.*	*Lb.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Cc*	*Cc*	*Cc*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
University Farm and Waseca [1]	1927	------	------	------	71	71	68–71	13	14	12–15	455	456	408–464	98	98	97–99	97	98	97–99
	1929	54	55	54–60	77	72	72–79	10	10	10–11	425	440	385–486	89	92	89–97	94	94	89–100
	1930	58	57	57–60	73	72	72–77	11	11	11–12	488	383	438–455	93	95	92–99	98	96	96–100
	1931	56	56	55–58	74	73	69–77	15	15	11–15	380	312	365–447	92	92	91–97	94	92	92–98
	1932	54	55	53–57	67	71	67–79	14	14	13–15	477	455	445–567	92	93	93–99	97	96	96–100
	1933	62	60	60–62	75	75	74–78	13	13	13–15	485	502	485–502	96	93	92–100	97	94	94–101

[1] Composite sample.

EXPERIMENTAL RESULTS

INTERANNUAL CORRELATIONS

The interannual correlations for each succeeding pair of years and the average correlations for all years are given in tables 2, 3, and 4 for each of the three groups of samples, namely, the spring wheat rod rows, the winter wheat rod rows, and the spring wheat 1/40-acre plots. The significant correlations are in bold-faced type in these tables. It should be kept in mind that stem rust was not serious enough to affect appreciably the relative placings of the varieties during the years included in this study. Under rust-epidemic conditions susceptible varieties are so altered that any normal interannual correlation with a nonepidemic season is completely eliminated.

It may be seen in these tables that there is a moderate tendency for varieties to hold their relative ratings for test weight from year to year. The original records showed that certain varieties, such as Reward, tended to have a high test weight, while Hope was low in this character.

TABLE 2.—*Interannual correlation coefficients for test weight, total flour, crude protein, absorption, loaf volume, and color, texture, and grain scores of loaf obtained from winter wheats grown in rod rows at University Farm and Waseca, Minn., 1927–33*

Years correlated	Comparisons	Interannual correlation coefficients							
		Test weight	Total flour	Crude protein	Absorption	Characteristics of loaf			
						Loaf volume	Color score	Texture score	Grain score
	Number								
1927, 1929 [1]	9	.....	−0.54	−0.23	−0.12	0.47	**0.81**	0.66	0.19
1929, 1930	14	0.26	**.78**	**.56**	−.47	**.60**	**.55**	.29	.15
1930, 1931	14	.58	.45	.27	.06	.37	**.88**	−.39	.09
1931, 1932	14	.30	.28	−.23	**.55**	−.30	**.74**	.48	.12
1932, 1933	7	−.27	.03	.65	.....	.63	.58	.40	.64
Average or total	58	[2] **.33**	**.37**	.20	[3] .03	**.32**	**.75**	.25	.19

[1] Crop failure in 1928.
[2] The number of comparisons in this case is 49.
[3] The number of comparisons in this case is 51.

TABLE 3.—*Interannual correlation coefficients for test weight, total flour, crude protein, absorption, loaf volume, and color, texture, and grain scores of loaf of spring wheat varieties grown in rod rows at University Farm, Waseca, Morris, and Crookston, Minn.*

Years correlated	Comparisons	Interannual correlation coefficients							
		Test weight	Total flour	Crude protein	Absorption	Characteristics of loaf			
						Loaf volume	Color score	Texture score	Grain score
	Number								
1927, 1928	11	.....	0.04	−0.10	−0.12	**0.81**	0.43	0.40	−0.18
1928, 1929	31	.....	−.16	−.10	−.10	**.38**	**.78**	.33	.12
1929, 1930	25	**0.58**	.27	**.48**	**−.41**	**.43**	**.78**	.26	−.29
1930, 1931	27	**.55**	**.45**	−.01	.27	.46	**.61**	**.60**	**−.48**
1931, 1932	16	.43	.44	.13	−.21	.15	**.82**	.15	.21
1932, 1933 [1]	13	.44	.19	.41	−.35	−.05	**.80**	.28	**.69**
Average or total	123	[2] **.52**	**.20**	.13	−.13	**.39**	**.74**	**.37**	−.06

[1] No data for Morris for 1933.
[2] Number of comparisons=81

TABLE 4.—*Interannual correlation coefficients for test weight, total yield of flour, crude protein, absorption, loaf volume, and color, texture, and grain scores of loaf of the spring wheats grown in 1/40-acre plots*

Years correlated	Interannual correlation coefficients [1]															
	Test weight		Total flour		Crude protein		Absorption		Loaf volume		Crumb color		Crumb texture		Crumb grain	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
1929, 1930	56	**0.55**	56	**0.60**	55	**0.70**	56	**0.31**	56	**0.35**	52	**0.58**	56	**0.42**	56	−0.01
1930, 1931	68	.23	68	**.50**	68	**.53**	68	.05	68	**.41**	51	**.51**	68	.24	68	−.09
1931, 1932	67	**.55**	67	.16	67	**.53**	67	.01	67	**.45**	52	**.50**	66	.19	67	**.42**
1932, 1933 [2]	36	**.55**	36	.26	36	.38	36	.18	36	.25	36	**.64**	36	.20	36	.13
Average or total	227	**.46**	227	**.40**	226	**.54**	227	**.15**	227	**.39**	191	**.55**	226	**.27**	227	**.12**

[1] *n*=Number of comparisons; *r*=correlation coefficient.
[2] No data at Morris in 1933.

There appears to be some basis for considering yield of total flour to be inherent, but the expression of this character is so obscured by random environmental effects that its usefulness is limited.

A similar situation holds with respect to crude protein content of the wheat. Although some varieties are consistently high or low in this character, many varieties appear to have a random type of variability.

In the group of baking scores there is one, crumb color, which appears rather definitely to be an inherent character because of the magnitude of its interannual correlations. This score is more nearly dependent upon one property of the wheat, namely, the carotinoid pigmentation, than are any of the other bread scores. In examining the individual interannual correlations of crumb color it should be kept in mind that in none of the years involved was there a serious epidemic of black stem rust that would have interfered with the expression of color. Interannual correlations for loaf volume were not large, although significant positive relationships were obtained for an average in each type of trial. There is little evidence of any inheritance of the properties of water absorption or of texture or grain of the loaf.

In a study of this type it must be kept in mind that there is considerable variation in both the response of the wheat plant to seasonal environmental factors and in the milling and baking procedure. When computing interannual correlations, therefore, the results of two highly variable tests are compared. It is not possible to grow a large number of varieties, so comparisons must be made between varieties grown in consecutive years. Higher correlations might have been obtained for some of the milling and baking characteristics if comparisons could have been made between averages of a considerable number of samples. It is possible and even probable that defects in the baking method used may have interfered with the proper expression of these characters. The possibility of this being the reason for the low interannual correlations is being investigated further at the Minnesota station.

INTERSTATION CORRELATIONS

The interstation coefficients of correlation for each of the characters included in this study were first calculated for each of the individual

years and then averaged to give the values in table 5. Only the spring wheat data from the 1/40-acre plots could be used, because the rod-row samples consisted of composites from the different stations. These correlations indicate that, while each variety tends to react in the same manner when grown in different locations, differences in soil and climatic factors greatly influence the expression of milling and baking characteristics. This fact suggests the desirability of studying the reactions of wheat varieties under as many conditions as practicable. The variation existing between the ripening dates for different varieties probably is an important cause of the low order of these correlations. In some cases it may be desirable to investigate methods of culture for experimental plots whereby the dates of maturity could be made to coincide for varieties as diverse as Marquis and Reward, in order to obtain comparable data on inherent characters.

TABLE 5.—*Interstation correlation coefficients for quality characteristics of spring wheat varieties grown in 1/40-acre plots at four stations in Minnesota, 1929–33* [1]

Stations correlated	Coefficients for average interstation correlation [2]															
	Test weight		Total flour		Crude protein		Absorption		Loaf volume		Color score [3]		Texture score		Grain score	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
University Farm and Waseca	81	**0.45**	81	0.17	80	**0.59**	78	**0.37**	81	**0.35**	66	**0.58**	79	**0.27**	79	**0.29**
University Farm and Morris	66	**.40**	66	.24	65	**.65**	67	**.42**	66	**.58**	34	.22	66	.20	66	**.46**
University Farm and Crookston	82	**.27**	82	.01	80	**.45**	79	**.33**	82	.23	67	**.63**	82	.27	80	**.37**
Waseca and Morris	65	**.68**	65	**.72**	64	**.60**	62	.24	65	**.27**	33	.11	65	**.28**	65	.20
Waseca and Crookston	81	**.37**	81	**.32**	80	**.40**	78	.23	81	.19	66	**.58**	79	**.30**	81	**.36**
Morris and Crookston	66	**.41**	66	**.29**	65	**.47**	63	.12	66	.09	34	.34	66	**.72**	66	**.48**
Total or average	441	**.44**	441	**.29**	434	**.52**	427	**.29**	441	**.29**	300	**.49**	437	**.35**	437	**.36**

[1] No data at Morris in 1933.
[2] *n*=number of comparisons; *r*=correlation coefficient.
[3] No color data at any of stations in 1929.

INTERRELATION BETWEEN CHARACTERS

The various coefficients of correlation between the characters for each of the three groups of samples are given in table 6. Some relations that have been found previously to be significant within varieties when grown under different environmental conditions do not appear to be of great importance when diverse varieties are included within the series and all varieties are grown under similar conditions. In this group are correlations of test weight with flour yield and protein content with crumb color. The small but significant positive correlations between protein content and loaf volume are in agreement, on the average, with the results of previous trials by other investigators.

The interlocking agreements between loaf volume, color, texture, and grain are as expected from long experience in test baking. This experience indicates that for a measure of color, texture, or grain the baking method should be so conducted as to yield bread of constant loaf volume, while for the determination of strength the volume should be pulled to a maximum. The results of these tests indicate some unsoundness in the underlying philosophy of baking-test methods now used that should be corrected as soon as methods can be developed.

TABLE 6.—*Interrelationships of the various milling and baking characteristics as expressed by the average correlation coefficients*

Characters correlated	Coefficients of intercharacter correlations [1]			
	Rod rows		¼o-acre plots of spring wheat	
	Winter wheat [2]	Spring wheat [3]		
	r	*r*	*r*	*n*
Total flour and test weight	**0.32**	0.06	**0.15**	309
Total flour and protein	.04	−.07	**.14**	309
Total flour and absorption	.22	−.05	.11	309
Total flour and loaf volume	**.39**	−.06	−.05	309
Total flour and color	.11	.03	.11	276
Total flour and texture	.04	**−.20**	.02	309
Total flour and grain	.12	−.01	−.06	307
Protein and absorption	.15	.08	.08	309
Protein and loaf volume	**.29**	**.16**	**.34**	309
Protein and color	**.43**	.15	.12	287
Protein and texture	**.25**	.13	**.29**	309
Protein and grain	−.06	**.16**	−.02	309
Absorption and loaf volume	.14	.05	.11	309
Absorption and color	−.17	**.28**	**.16**	287
Absorption and texture	−.12	**.24**	**.21**	309
Absorption and grain	−.12	.15	.04	307
Loaf volume and color	**.32**	**.25**	**.22**	287
Loaf volume and texture	**.25**	**.23**	**.65**	309
Loaf volume and grain	**.60**	**.27**	.03	307
Color and texture	**.50**	**.56**	**.63**	287
Color and grain	**.42**	**.35**	**.44**	264
Texture and grain	**.26**	**.42**	**.37**	307

[1] *r*=Correlation coefficient; *n*=number of comparisons.
[2] Number of comparisons=88.
[3] Number of comparisons=188 except for total flour and test weight, where *n*=156.

DISCUSSION

New varieties of wheat produced by the wheat breeder not only must have desirable agronomic characters and resistance to the more important plant diseases but they must also excel or equal in milling and baking qualities the best varieties grown commercially. These milling and baking qualities depend (1) on the inherent qualities of the varieties and (2) on the influence of environmental factors on these inherent qualities. At present there is no method available for the determination of the quality of new varieties during the early segregating generations. If such a method could be evolved it would greatly simplify the plant breeder's problem.

In the absence of such a method, however, it is necessary to mill and bake a large number of samples of the later generations when sufficient grain becomes available from the material grown under a normal range of environmental conditions. These milling and baking tests are made after the new strains have progressed to the rod-row plots. A composite sample of seed obtained from trials made in different localities within the State is used. The data obtained from such tests are used to aid in selecting the more promising wheats and in discarding the less desirable ones. Later, as more seed becomes available, varieties as grown in each locality may be milled and baked.

The most striking feature of the milling and baking data on these wheats was the relatively low magnitude of the observed interannual coefficients of correlation for most characters. The outstanding exceptions to this low order of correlation were crumb-color scores. It would appear that the relative placing of wheat varieties for this character was less affected by environmental factors than was

the placing for any other character. However, in the placing for crumb color other factors tended to interfere with this relation. To some extent, as indicated by the numerous significant correlations, the crumb color was partly a function of the loaf volume and of the nature of the crumb of the cut loaf. From a plant-breeding standpoint, therefore, it would seem necessary to make tests for color of flour in the early stages, and methods are available for making these determinations.

The milling yield as expressed in percentage of total flour secured from the wheat tended to be independent of all the factors that affect the baking results. The interannual average correlations for total flour were usually significant and positive but of a low order. The same was true of the interstation correlations, with the exception of the high correlation of $r=0.72$ for Morris and Waseca. It has been shown by many workers, as well as by the accumulated knowledge of practical millers, that the milling yield is largely a function of the test weight of the wheat, although correlation coefficients of low order were obtained in this study. The test weight of the wheat has been shown repeatedly to be reduced by such factors as moisture deficiency in the soil during the ripening period, hot and desiccating atmospheric conditions, frosts, and attacks of diseases such as rusts and root rots.

During the years of these trials the test weights were frequently adversely affected by one or more of these factors with the exception of frost. The varieties used had different dates of ripening, there being a range of over a week between the earliest and the latest varieties. Under these conditions it is not surprising that there were not high interannual and interstation correlations of milling yield. The milling yield should never be considered without keeping in mind at the same time the test weight of the wheat and the kernel texture.

Crude protein content is often rather closely correlated with loaf volume when commercial samples are compared. In previous studies with wheats of diverse nature, grown under similar environmental conditions, Hayes et al. (*6*) found little evidence of consistent correlation between loaf volume and crude protein. In the present studies, carried over many years, there was opportunity to draw more accurate conclusions.

Interannual correlations for crude protein content and loaf volume for rod rows of winter wheat were 0.20 and 0.32, respectively, the latter reaching the 5-percent point; for spring wheat rod rows, 0.13 and 0.39, respectively, the latter reaching the 5-percent point; in the $\frac{1}{40}$-acre plots of spring wheat both correlation coefficients were significant, being 0.54 and 0.39 respectively.

In interstation correlations during the same years, the coefficients for crude protein exceeded the 5-percent point in each case and ranged from 0.40 to 0.65. With loaf volume all six correlations were positive, three reaching the 5-percent point. These facts lead to the conclusion that inheritance is of considerable importance for both crude protein and loaf volume but that environmental conditions tend strongly to modify the results of individual comparisons.

When data of all tests are combined, giving n values of 88, 188, and 309, the correlations between crude protein and loaf volume are 0.29, 0.16, and 0.34, respectively, which certainly are of little selection value although they do show positive relationship.

The baking test is an extremely complicated biochemical and biophysical process. Because of its complexity and sensitivity to environmental influence, it is fully as difficult to control for scientific purposes as is the growing of the varieties of wheat in truly comparable tests by the agronomist. Test-baking procedures may be classified in two fundamentally different categories. One includes those tests that subject all samples to a more or less rigid technique and set of conditions, the other includes those that tend to give each sample its optimum treatment. At present there is no evidence that conclusively proves one of these categories to be superior to the other for wheat-variety testing. The method used in the studies here reported was the rigid procedure for all varieties. Thus the variations observed could be said to be functions of the variety or station or year rather than of the method. These variations might or might not have been observed if some other baking technique had been employed. The milling and baking tests for the years 1927 and 1928 were conducted by one technician, and all samples from 1929 to 1933, inclusive, were milled and baked by a second technician. In this way personal variability was held close to a minimum.

The various scores of the bread tended to be correlated, but most coefficients were rather small. In the winter wheats the correlation of loaf volume and crumb grain was $r = 0.60$, while in the spring wheats from the $\frac{1}{40}$-acre plots it was very low, being $r = 0.03$. In the winter wheats the grain scores closely followed the loaf volumes, but the spring wheat loaves exhibited a wide range in grain scores at each loaf-volume level. Some varieties, such as Marquillo, tended to give a coarse, open grain regardless of volume. Certain other varieties of questionable gluten strength gave very poor grain with a large volume. Other varieties, such as Marquis, gave uniformly good grain at widely varying levels of loaf volume. Loaf volume and crumb texture were found to be correlated; this was especially noticeable on some of the spring wheat flours from the rod-row series in which the volume was large and the texture very soft and silky. Crumb color was correlated with texture and grain. This was due to shadows cast by the cell walls, making coarse-grained bread darker in color than the fine, even-grained type. This relation did not hold in the case of extremely tight-grained loaves, because the thickness of the cell walls gave undue yellowness to the appearance of the slice.

SUMMARY

Milling and baking tests were made on varieties of wheat grown as a part of the wheat-breeding program during the years 1927–33, in the rod-row and $\frac{1}{40}$-acre varietal trials of the Minnesota Agricultural Experiment Station.

Correlation analysis was used to determine interannual, interstation, and intercharacter relationships for the characters of test weight, wheat protein, milling yield of straight grade flour, water absorption of the dough, loaf volume and color, and texture and grain of the cut crumb of the loaf.

The magnitude of the interannual correlations tended to be rather low with the exception of crumb color, which appeared to be more independent of interfering environmental variables than did the other factors.

Interstation correlations tended to be significant but of a low order of magnitude, indicating a need for further study of the environmental factors that affect milling and baking quality and the desirability of developing practical methods of handling experimental plots so that all varieties will mature at the same time instead of with a spread of a week or more as at present.

Intercharacter correlations indicate a close relation between loaf volume and color, texture, and grain. The relation between test weight, protein content, and milling yield was not apparent when varieties of diverse origin were compared.

Better methods for determining the baking quality of wheat varieties are very urgently needed and should be based upon sound biological and physical studies.

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CORRELATION OF BODY MEASUREMENTS OF SLAUGHTER STEERS WITH RATE AND EFFICIENCY OF GAIN AND WITH CERTAIN CARCASS CHARACTERISTICS [1]

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INTRODUCTION

As a part of record-of-performance work at the Agricultural Research Center, Beltsville, Md., the cattle used in such work were measured at the time of slaughter to determine whether any relationship existed between body measurements and various production factors and meat characters. The record-of-performance procedure necessitated that all the animals be of approximately the same weight (*1, 10*).[3] For this reason, the data are for a nearly weight-constant population. The variation in slaughter weight was about ±20 pounds from a mean of 900 pounds live weight. The results of the correlation study are reported herein.

REVIEW OF LITERATURE

In a study of type in beef calves, Hultz (*5*) showed that the most important measurements for the determination of low setness are depth of chest, paunch circumference, and height at withers. In a study made at time of slaughter, Hultz failed to obtain a significant correlation between any of the body measurements of the calves and their type. The highest correlation he obtained was between the ratio of chest depth to height at withers and type. This correlation was too low to be significant.

In a study of type in 2-year-old steers, Hultz and Wheeler (*6*) showed that a steer has the appearance of being low-set when the proportions of his chest depth, heart girth, and paunch girth to height are great. In other words, a low-set steer has a large heart girth, is deep of chest, and has a large paunch girth in proportion to the height at withers.

Lush (*8*) obtained a correlation of 0.563 between estimated fatness and a ratio of heart girth to height at withers of the steer. This is the highest correlation with estimated fatness obtained. The ratio of loin width to heart girth gave a correlation of 0.076. He concluded that ratios involving chest width have higher correlations, larger average changes due to fattening, and larger standard deviations of the changes than ratios involving loin width. This finding seems to indicate that width of chest is more closely related to fatness than width of loin but was not measured so accurately as width of loin.

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[2] Acknowledgment is made to O. G. Hankins and R. L. Hiner, of the Animal Husbandry Division, for making the meat studies and to L. B. Burk, A. T. Edinger, and M. T. Foster, of the Bureau of Agricultural Economics, for cooperation in grading the slaughter cattle.
[3] Reference is made by number (italic) to Literature Cited, p. 472.

In a study of the relation of body shape of feeder steers to rate of gain, to dressing percentage, and to value of dressed carcass, Lush (*9*) showed that for high dressing percentages and dressed-carcass values, the most important measurements are a large heart girth in connection with a shallow chest, a wide loin, and large flank girth, a large initial weight, small paunch girth, head narrow at the eyes, and a short height over hips. Maximum gains, Lush concluded, are associated with a long body, tall at the withers, with a large paunch girth but small flank girth and narrow at the loin—a big skeleton but poor in flesh.

Knapp and Cook (*7*) demonstrated that between beef and dual-purpose Shorthorns certain differences exist in body measurements and in ratios of body measurements. According to their data, with steers slaughtered at 900 pounds weight the animals with the lowest height at withers are of the better beef type. In a study of the ratio of height at withers to heart girth they showed that there is a significant difference between beef and dual-purpose cattle.

MATERIAL AND METHODS

The 50 animals used in this study were record-of-performance steers of beef, dual-purpose, and dairy breeding. They were fed individually corn, oats, wheat bran, and linseed meal before weaning and corn and alfalfa hay after weaning. All animals were weaned at 252 days of age. As already stated, the population studied was nearly weight constant, the steers being slaughtered at as nearly 900 pounds live weight as possible.

A committee consisting of three men familiar with the standard market grades of steers, as adopted by the United States Department of Agriculture, graded the slaughter steers. Figure 1 shows the position of all the measurements taken on an animal.

Correlations were obtained between the various body measurements, or ratios of body measurements (shown in table 1), and efficiency of gain, average daily gain, dressing percentage, percentage of fat in carcass, percentage of total edible meat in carcass, and slaughter grade. The correlations were computed according to the methods of Wallace and Snedecor (*11*). Yapp's index is computed as outlined in his paper (*12*). This index takes into consideration height, length, and weight of the animal. No standard error was computed, but, according to Fisher (*4*), for a P of 0.05 based on a sample of 50, the correlation, to be significant, must be higher than 0.273, and for a P of less than 0.01 the correlation must be higher than 0.354. Thus, in the following results, correlations higher than 0.354 are considered significant, whereas those between 0.273 and 0.354 are considered of questionable significance.

RESULTS

In table 1 are shown the positive and negative correlations between the various body measurements and factors of beef production.

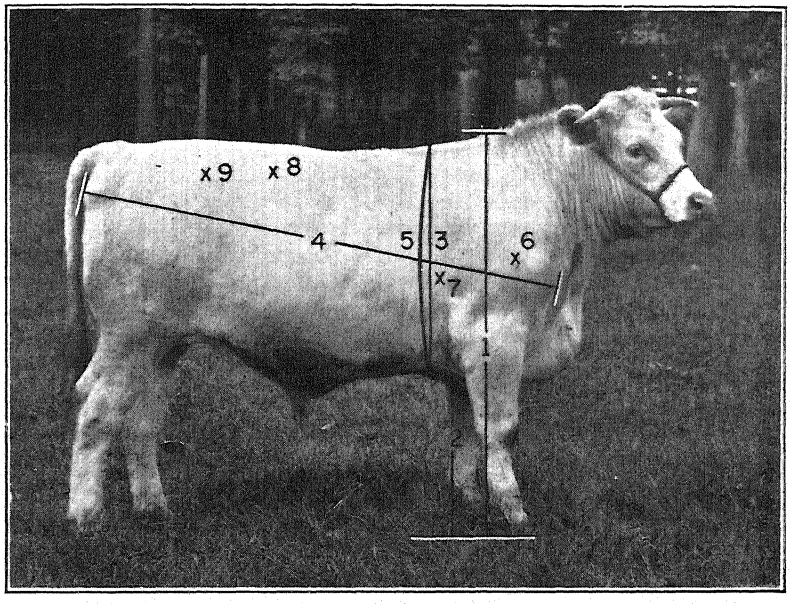


FIGURE 1.—Location of measurements studied: 1, Height at withers; 2, height of floor of chest; 3, depth of chest; 4, length of body from pinbone to shoulder point; 5, heart girth; 6, width of shoulder; 7, width of chest; 8, width of loin; and 9, width of hips. (× indicates measurements taken through body.)

TABLE 1.—*Correlations of body measurements and ratios of body measurements with the factors indicated, in record-of-performance steers*

Body measurements	Efficiency of gain	Average daily gain	Dressing percentage	Percentage of fat in carcass	Percentage of total edible meat	Slaughter grade
Height at withers	−0.3667	−0.1907	−0.5011	−0.7989	−0.8296	−0.8293
Height of floor of chest	−.2909	−.1252	−.4602	−.6974	−.6762	−.7105
Depth of chest	−.2425	−.1871	−.3857	−.4613	−.5467	−.5290
Length of body	−.4335	−.3224	−.4875	−.6347	−.7116	−.7447
Heart girth	+.0721	−.0542	+.1283	+.2969	+.2240	+.3908
Width of shoulder	+.4504	+.4212	+.3890	+.4113	+.4447	+.6144
Width of chest	+.3390	+.2929	+.5019	+.5844	+.5769	+.6605
Width of loin	−.0517	−.0527	−.1104	−.1557	−.0973	−.0572
Width of hips	−.0910	−.2002	+.1904	+.2509	+.2094	+.3585
Ratio of heart girth to height	+.3226	+.1216	+.5356	+.7611	+.7600	+.8252
Yapp's index	−.4430	−.2850	−.5659	−.7632	−.7934	−.8593
Ratio of body weight to height	+.4407	+.3323	+.5320	+.7537	+.7668	+.8361
Heart girth × 100 / Height × length	+.4063	+.2301	+.5631	+.7736	+.8072	+.8713
Ratio of width of chest to height at withers	+.4042	+.2900	+.6144	+.7362	+.7498	+.8145
Slaughter grade	+.4962	+.3541	+.6431	+.8201	+.8325	

CORRELATIONS WITH BODY MEASUREMENTS

Height at withers is, strictly speaking, a skeletal measurement and shows a negative correlation with all the factors studied. The correlation between height at withers and efficiency of gain is significantly negative. The taller, rangier type of animal, therefore, has a slight tendency to put on gains less economically than the shorter, blockier type. However, there was no significant correlation between height at withers and rapidity of gain. According to the results obtained, the shorter type steers have the higher dressing percentage; the rangier,

taller animals are lower in this respect. This correlation is significant. Height at withers was highly correlated negatively with percentages of fat and edible meat in the carcass. The taller type animals, therefore, have less fat and less total edible meat than the lower, blockier type. There is a very high negative correlation between height at withers and slaughter grade.

Length of leg, as shown by height of floor of chest, has a negative correlation with the production factors considered. The correlations are not so high throughout as those involving height at withers, but they are in about the same order. The correlation between height of floor of chest and efficiency of gain is of questionable significance, whereas that between height of floor of chest and average daily gain is not significant.

Depth of chest appears to be a skeletal measurement in that, like other skeletal measurements, the correlations are all negative. Again the correlations are slightly lower than those involving height at withers. From the three measurements so far discussed, it can be concluded that with weight constant, the shorter in height, shorter legged, shallower type of animal is higher in efficiency of feed utilization and has more fat, more edible meat, and less bone in the carcass than the taller, longer legged, deeper bodied animal. This is in agreement with the findings of Lush (*9*).

Length of body, as measured from pinbone to shoulder point, shows higher correlations with efficiency of gain and with rapidity of gain than does height at withers but lower correlations with dressing percentage, percentage of fat, and percentage of edible meat. The correlation between height at withers and length of body is 0.6957. The fact that the coefficient of alienation equals the coefficient of correlation does not necessarily indicate that an animal short in height is short in body. The actual length of body shows considerable variation from the estimated length derived from the actual height at withers, computed by the estimating equation from the correlation of 0.6957. In other studies made by the writers, length of body had a much higher correlation with efficiency of gain than did height at withers. In the present study the difference is not significant. It may be concluded, however, that length of body is associated more closely with efficiency of gain than is height at withers.

As shown by table 1, negative correlations were obtained between all the skeletal measurements and the quality of the end product, as judged by the quantity of meat or fat or the slaughter grade of the animal. This result is to be expected in a nearly weight-constant population.

Heart girth shows only two correlations of even questionable significance—those involving percentage of edible meat and slaughter grade. With a weight-constant population, it is not expected that there would be any correlation between heart girth and the remaining four factors. Heart girth has been used to determine weight of animal, and the relationship between these two factors is very close. The positive correlation between heart girth and slaughter grade shows that condition of fleshing plays a part in size of heart girth for a given weight.

Width of shoulder and width of chest show positive relationships with all the factors studied. The correlations involving width of

shoulder are consistently higher for efficiency of gain and lower for the characteristics of the slaughter animal than the correlations involving width of chest.

Width of loin is not correlated with any of the factors. The correlations are consistently negative but none may be considered significant. This finding is contrary to that of Lush (*8*), who shows that width of loin makes next to the largest change with increasing fatness. Lush's data were not used for a weight-constant population.

Width of hips shows low insignificant negative correlations with efficiency and rapidity of gain and low positive correlations with slaughter grade, percentage of fat, percentage of edible meat, and dressing percentage of the animals. The only correlation that may be considered significant is between width of hips and slaughter grade.

CORRELATIONS WITH RATIOS OF BODY MEASUREMENTS

The correlations of ratios of body measurements with production factors are rather high when compared with the results obtained by Lush (*8*) and by Hultz (*5*, *6*). This lack of agreement may be caused by the fact that the population studied by the present authors was a weight-constant group, whereas the populations studied by Lush and Hultz were not.

The ratio of heart girth to height at withers has a significant correlation with dressing percentage, percentage of edible meat, percentage of fat, and slaughter grade but not with either efficiency of gain or rapidity of gain. The correlation of 0.76 with percentage of edible meat in the carcass shows that this ratio may be used as a method of predicting percentage of total edible meat. A well-finished steer, therefore, should be less tall for a given circumference of chest than a poorly finished steer.

Since there were certain slight deviations in weights of the animals at time of slaughter, a ratio of weight to height at withers was computed. The correlations show that it is a better measure of slaughter grade, percentage of edible meat, and efficiency of gain than is the ratio of heart girth to height at withers. These correlations are about the same as those obtained with height at withers alone, except that one group is positive and the other negative.

Yapp's index, although negative, shows in respect to efficiency and carcass characteristics, with the exception of dressing percentage, the highest correlations of the group of ratios. Only one of the correlations, average daily gain, is of doubtful significance; the others are highly significant. This index may be used to estimate percentage of fat and percentage of total edible meat but is not so efficient as the judgment of an experienced grader. Percentage of fat may be estimated from slaughter grade with 9 percent less error, and percentage of total edible meat with 6 percent less error, than from Yapp's index. The fact that weight is constant may account for higher correlations than Yapp has shown. The difference in correlations between Yapp's index and the weight-height ratio is very small, and because of the number of measurements required in the Yapp index it is not believed that it is a much better measure.

The ratio $\frac{\text{heart girth} \times 100}{\text{height} \times \text{length}}$, suggested by Duerst (*3*), is similar to

the Yapp index. The correlations are higher than those shown for the ratio of heart girth to height at withers, and in most instances are superior to those obtained by the weight-height ratio. The ratio $\frac{\text{heart girth} \times 100}{\text{height} \times \text{length}}$ shows a higher correlation with slaughter grade, percentage of fat, and percentage of edible meat than any of the other ratios. The correlation with slaughter grade of 0.8713 is high enough to be of use in determining the dressing percentage, percentage of fat, and percentage of edible meat of an animal. Likewise, the high correlation with percentage of total edible meat makes possible the use of this ratio to estimate percentage of total edible meat. However, it is not so easy to use as the ratio of body weight to height at withers, and the difference is not great enough to warrant the time required to measure anything except height at withers.

The ratio of width of chest to height at withers is in fair agreement with the other ratios. As a whole, this ratio does not show as high correlations as the other ratios. The correlations with efficiency of gain, average daily gain, and dressing percentage are higher than those obtained by the use of the ratio of heart girth to height at withers, whereas the correlations of percentage of fat, percentage of edible meat, and slaughter grade are lower than those obtained by this ratio.

Slaughter grade shows throughout higher correlations than any of the ratios. That visual observation should be superior to measurements or ratios of measurements seems reasonable since measurements cannot show exactly the symmetry and proportions that should exist in a good beef-type animal. All the correlations obtained through the use of slaughter grade are significant. The correlation of 0.8325 with percentage of edible meat in the carcass shows that selections are being made of animals with low percentage of bone. Taken as a whole, the correlations indicate that committee judgment is a more accurate estimate of the worth of an animal than any ratios of body measurements. Lush (*8*) concludes that measurements should be regarded as supplementary and confirmative of the inferences drawn from weight and appearance rather than as a substitute for the information gained from the study of the weights alone. The authors have likewise found it to be true in regard to slaughter grade of animal; measurements can well be a part of such studies but should not be substituted for slaughter grade of the animal.

The authors found that when weight of the animals is kept constant there is a much higher relationship between body measurements and ratios of measurements and various production factors than either Lush or Hultz found in their studies. A comparison of the ratios and their correlations shows that Yapp's index, in general, gives the highest correlations with efficiency of gain and with the carcass characteristics studied. The weight-height ratio gives almost as high correlations and for all practical purposes they may be considered the same. The heart girth-height ratio on an average gives the poorest correlations in the group of ratios, although the correlation with slaughter grade is high.

To make use of these ratios a study must be made of changes occurring in the ratios during the life of an animal; in other words, changes in the ratios due to increasing fatness, weight, and age. The ratio of weight to height, for instance, changes gradually from about

1.2: 1 at birth to 7.5: 1 at 15 months of age, as found by the authors in a study with Shorthorn steers, the number in the various groups ranging from 6 to 23. The results agree in general with those of Brody and his coworkers (*2*), who used Hereford and Shorthorn steers (fig. 2). These findings show that if this ratio is to be of use in a random-weight population, an age correction factor must be employed. The important point is that the ratio between any one of the skeletal measurements and weight changes with age as well as with fatness.

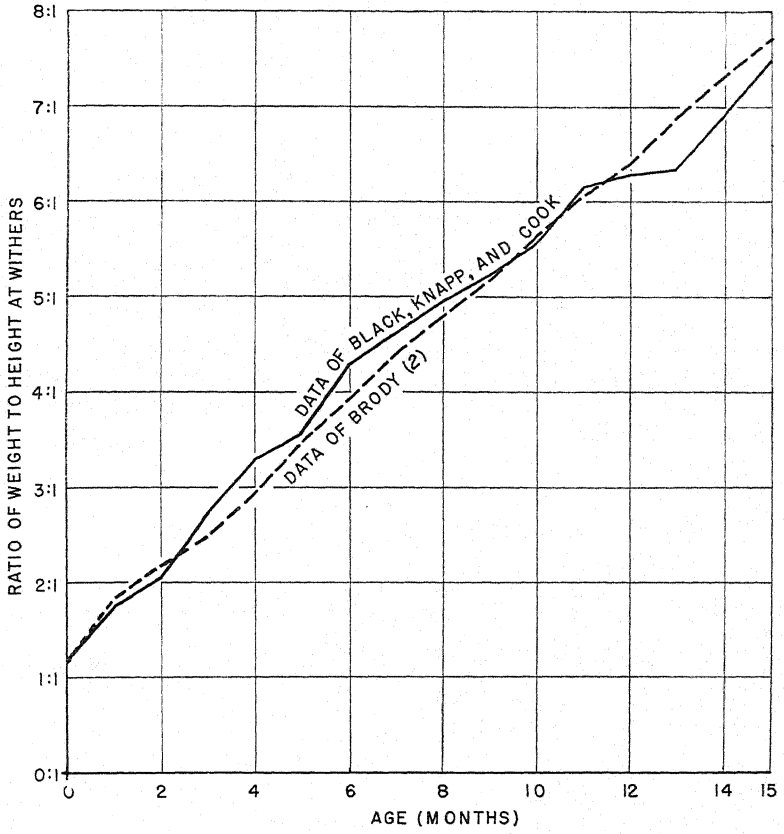


FIGURE 2.—Effect of age of steers on ratio of weight to height at withers.

The recent trends in beef type have been to produce the most meat with the least skeletal growth. Such a trend should not go to the extreme in small skeletal growth.

SUMMARY

A correlation study was made of the body measurements, in relation to efficiency of gain, average daily gain, dressing percentage, percentage of fat in carcass, percentage of total edible meat, and slaughter grade, of 50 head of record-of-performance steers slaughtered at the Agricultural Research Center, Beltsville, Md. They were of beef, dual purpose, and dairy breeding. All animals were slaughtered at

Correlations indicate that height at withers (with weight nearly constant) is one of the best measurements of performance, although length of body had a higher correlation with efficiency of gain and average daily gain than did height at withers.

Of the ratios of measurement studied, a ratio of weight to height at withers gave a higher correlation with performance factors than any other ratio for the number of measurements taken. Yapp's index, which takes into consideration the height, weight, and length of the animal, gives in general the highest correlations. However, the differences between them and the correlations obtained by the use of the weight-height index are not sufficiently great to justify taking the large number of measurements required in the use of Yapp's index.

Slaughter grade was found to be a better measure of beef type than any ratios of measurements. Therefore, measurements should not replace, but should supplement, reliable slaughter grades.

If any ratio is used, corrections will have to be made for differences in fatness, weight, and age of the animal, for these ratios do not remain constant for any one individual during the entire course of its life.

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SPOTTING OF FIGS ON THE MARKET [1]

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INTRODUCTION

Figs (*Ficus carica* L.) that are poorly refrigerated in transit or that are held on the market after arrival are likely to develop a surface spotting that seriously detracts from their market value. This spotting occurs on fruit from the Atlantic as well as on that from the Pacific coast and on all varieties that have been under observation. Smith and Hansen [3] have described a disease that appears to be identical with that under consideration and have reported its occurrence in the San Joaquin Valley of California both on the fruit on the tree and on fruit in transit to the cannery. They found *Alternaria* sp. to be the causal organism.

DESCRIPTION AND CAUSE OF THE DISEASE

The spotting is confined largely to fully ripe fruit and is favored by cracks in the skin and by the sugary solution often found on the surface of the fig. Species of *Alternaria*, *Cladosporium*, and *Botrytis* have been isolated from the diseased areas, but *Alternaria* has been the prevailing fungus.

ALTERNARIA

The *Alternaria* spots are first evident as small grayish-white tufts of mycelium; as these enlarge they soon darken to an olivaceous color [4] (fig. 1). At first the fungus appears to have but little attachment to the skin and sometimes can be rubbed off with little evidence of injury; but as the spots enlarge and become slightly sunken, any attempt to remove the fungus also removes the skin. The organism apparently can penetrate the skin readily. The mycelium is soon found in abundance at a depth of several cells with scattering hyphae much deeper in the tissue (fig. 2).

The foregoing description applies particularly to the *Alternaria* spotting on California figs. Figs from Norfolk, Va., have a similar spotting due to *Alternaria*, but the fungus retains its whitish-gray color longer and never becomes as dark as the California fungus (fig. 3).

The two forms of *Alternaria* also show differences in growth on culture media (figs. 4, 5, 6, 7). In Petri-dish cultures the Virginia *Alternaria*, unlike the California form, often develops a radial appearance and a hairy, threadlike growth, and on water agar it shows a tendency to coil (fig. 7).

[1] Received for publication August 26, 1937; issued May 1938.

[2] The authors are indebted to W. T. Pentzer and C. E. Asbury, of the Division of Fruit and Vegetable Crops and Diseases, for cooperation in obtaining the figs from Fresno, Calif., and to C. O. Bratley, of the same Division, for cooperation in obtaining those purchased on the New York market.

[3] SMITH, R. E., and HANSEN, H. N. FRUIT SPOILAGE DISEASES OF FIGS. Calif. Agr. Expt. Sta. Bull. 506, 84 pp., illus. 1931.

[4] This and later color references are based on Ridgway standards. RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.

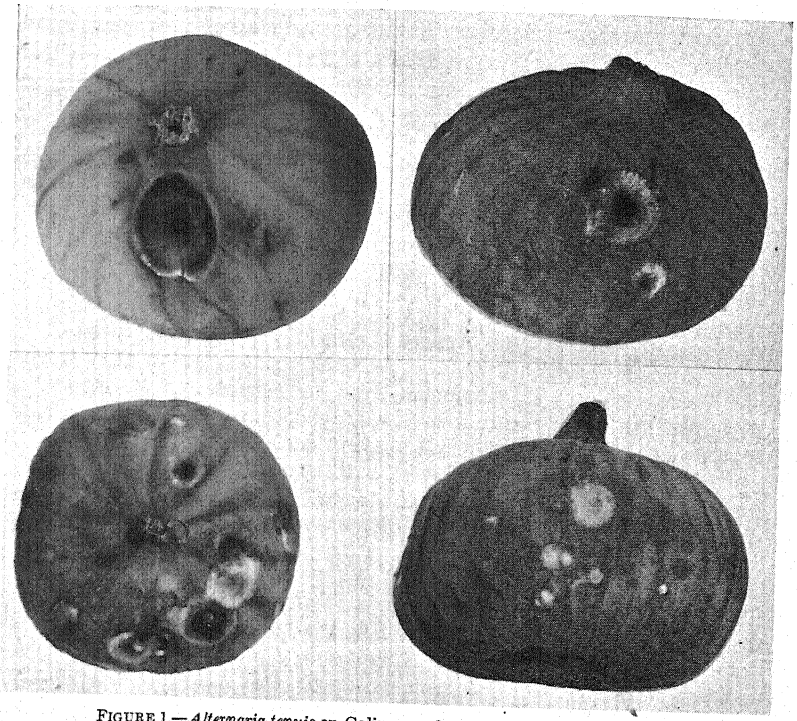


FIGURE 1.—*Alternaria tenuis* on Calimyrna figs as received from California.

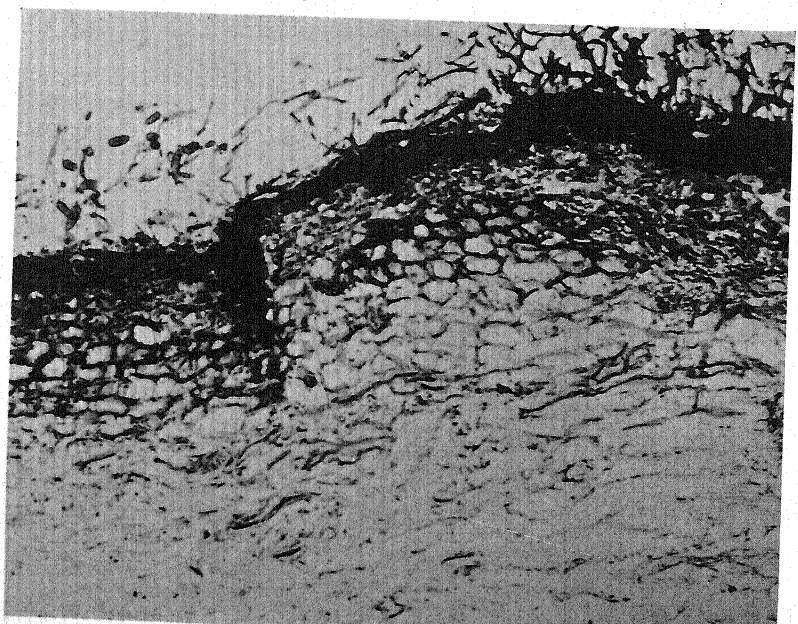


FIGURE 2.—Photomicrograph of an *Alternaria* lesion from a California Black Mission fig as seen in radial section. × 140. Note the spores and mycelium on the surface, the thick network of hyphae between the outer layers of cells, and the occasional hypha at a depth of 15 or 20 cells.

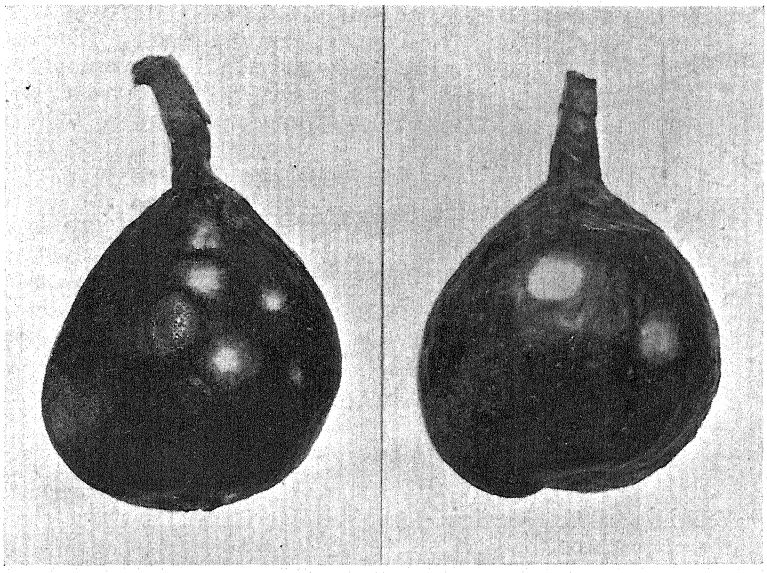


FIGURE 3.—Spotting on California Black Mission figs resulting from inoculation with the *Alternaria* isolated from Norfolk, Va., figs.

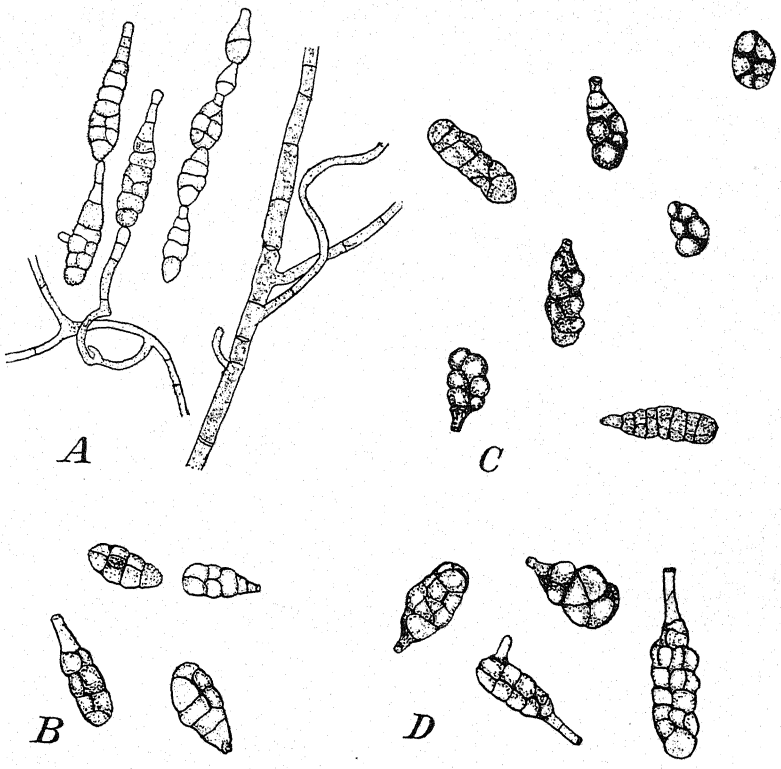


FIGURE 4.—Conidia of the California *Alternaria*. *A*, From a 30-day culture on water agar; *B*, from California figs; *C*, secondary characters of conidia from a culture on potato-dextrose agar; *D*, secondary characters of conidia from an inoculation on figs. × 450.

The following measurements and studies are based largely on water-agar cultures. The scant mycelial development on this medium gives an excellent opportunity to follow the spore formation, and the spores and sporophores are apparently typical.

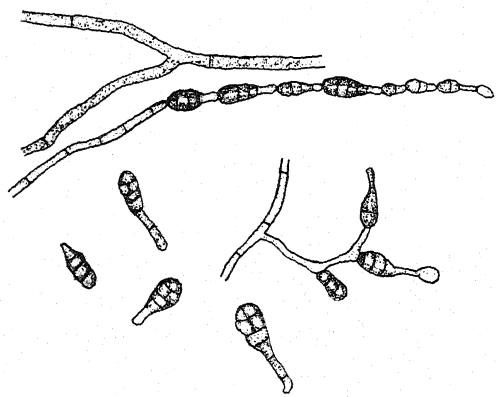


FIGURE 5.—Conidia of the Virginia *Alternaria* from a culture on water agar. × 450.

The conidiophores are darker than the mycelium, and they may be either erect or decumbent. On the California *Alternaria*, they were 25μ to 250μ by 3μ to 5μ and were sometimes moderately echinulate; on the Virginia *Alternaria*, they were 20μ to 180μ by 4μ to 5μ and echinulation was not observed.

The conidia of the Virginia form were produced in short chains, having usually three to four and sometimes as many as nine conidia to a chain. They were obclavate to pyriform, muriform; sometimes echinulate; at first pale yellow green but later olivaceous; one- to five-septate; and measured 17μ to 35μ by 6μ to 10μ.

The conidia of the California form were produced in long chains that often developed secondary branching. The conidia were obclavate to pyriform and broadly muriform; definitely echinulate; at first pale to amber yellow but later olivaceous to fuscous; usually short-beaked; longitudinally and transversely septate; and measured 20μ to 75μ by 8μ to 17μ. The development of secondary character was found on all substrata tested, the conidia finally became much distorted and swollen, and septa formed at various angles.

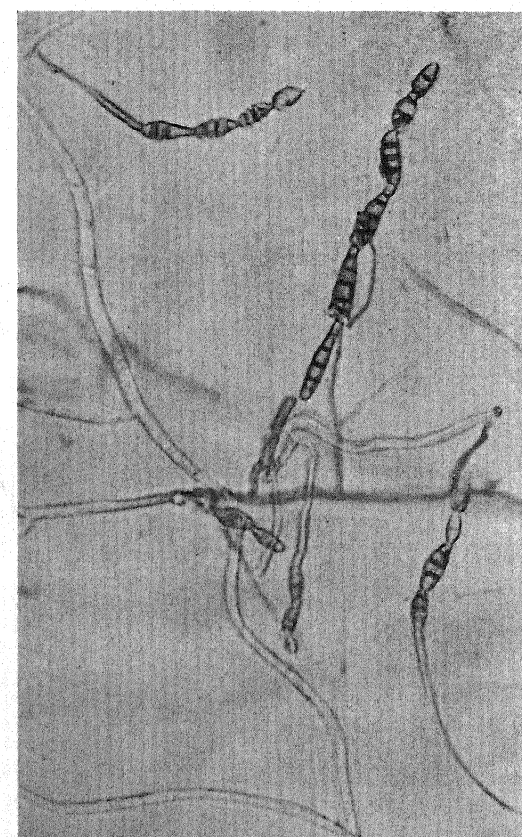


FIGURE 6.—Photomicrograph of the California *Alternaria* after 4 days on water agar. × 270.

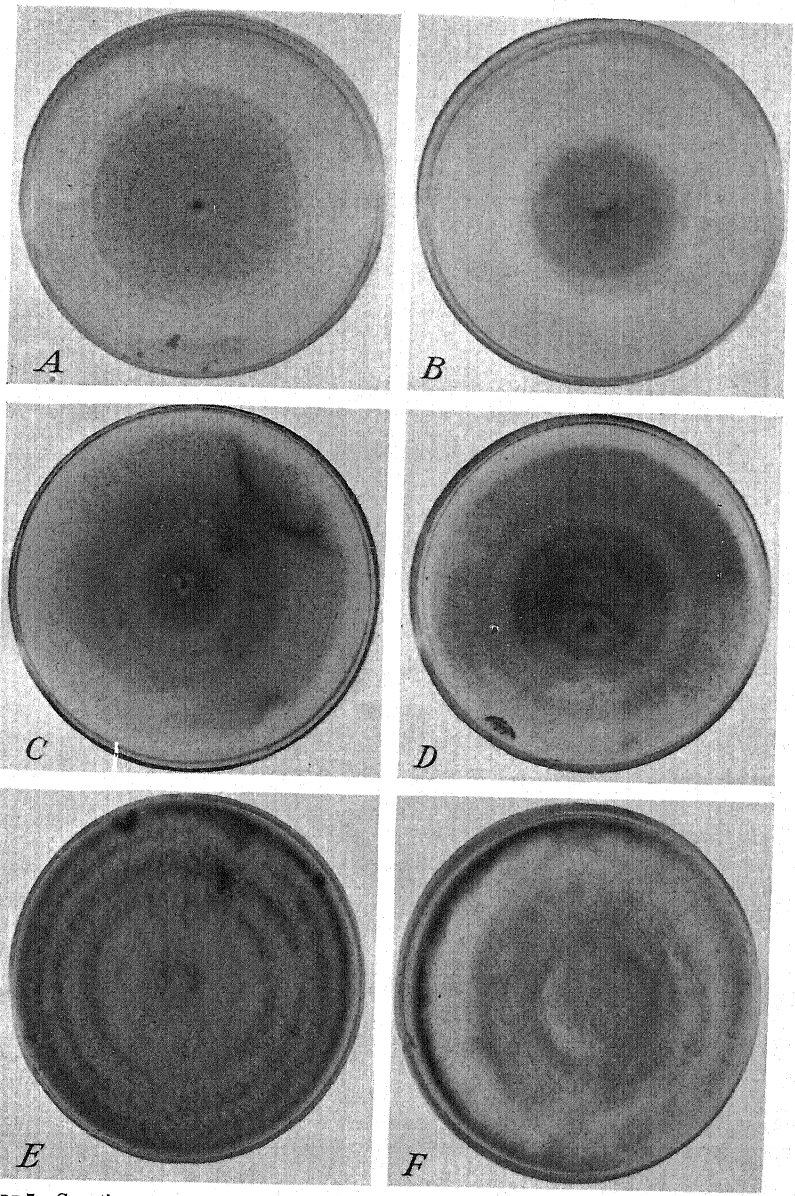


FIGURE 7.—Growth contrasts of the California *Alternaria* and the Virginia *Alternaria* after 8 days at room temperature: *A*, California *Alternaria* on water agar; *B*, Virginia *Alternaria* on water agar; *C*, California *Alternaria* on corn-meal agar; *D*, Virginia *Alternaria* on corn-meal agar; *E*, California *Alternaria* on fig agar; *F*, Virginia *Alternaria* on fig agar.

The California organism is readily included in the *Alternaria tenuis* group as described by Elliott,[5] and there does not seem to be sufficient ground for excluding the Virginia organism from this group. Until further studies of the genus are made it seems best to consider the two fungi as strains or varieties of *A. tenuis* Nees.

CLADOSPORIUM

A *Cladosporium* isolated from spots on California figs was found to have some pathological significance. Figure 8 shows the type of spotting produced on Calimyrna figs after inoculation with a spore suspension of this fungus.

The *Cladosporium* spots appear first as dark olive-green specks that are particularly noticeable on the light-skinned varieties. The

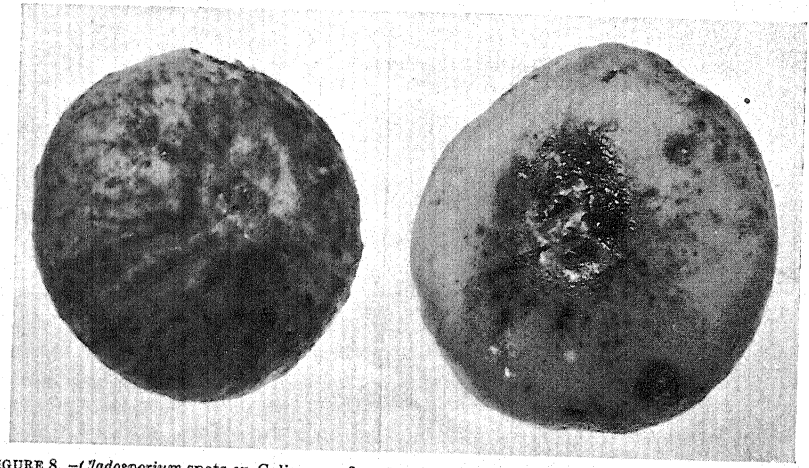


FIGURE 8.—*Cladosporium* spots on Calimyrna figs after inoculation with a spore suspension and prolonged holding at 32° F.

lesions enlarge, become slightly depressed, and turn to a yellowish-olive color.

Conidial production begins early, and the surface growth on the fruit is composed largely of conidiophores and conidia. The conidiophores arise in tufts, erect or nearly so, from a nodular mass of cells embedded in the epidermis. They are septate and brownish olive in color and are 3μ to 4.8μ in diameter.

The conidia take various forms—cylindrical, elliptical, ovoid, subfusiform, and subglobose. All are continuous at first but later the basal and immediately adjacent conidia may become once or more septate (fig. 9). Occasionally other conidia become once septate. As the conidia mature they change from hyaline to pale greenish yellow and finally become olivaceous. Excluding the elongated basal cells, the conidia measure 3μ to 7.2μ by 2μ to 5.6μ. The basal cells, which are readily detached as conidia, measure 12μ to 24μ by 3.6μ to 5μ. The conidial forms in culture were similar to those found on the fruit.

[5] ELLIOTT, J. A. TAXONOMIC CHARACTERISTICS OF THE GENERA ALTERNARIA AND MACROSPORIUM. Amer. Jour. Bot. 4: 439–476, illus. 1917.

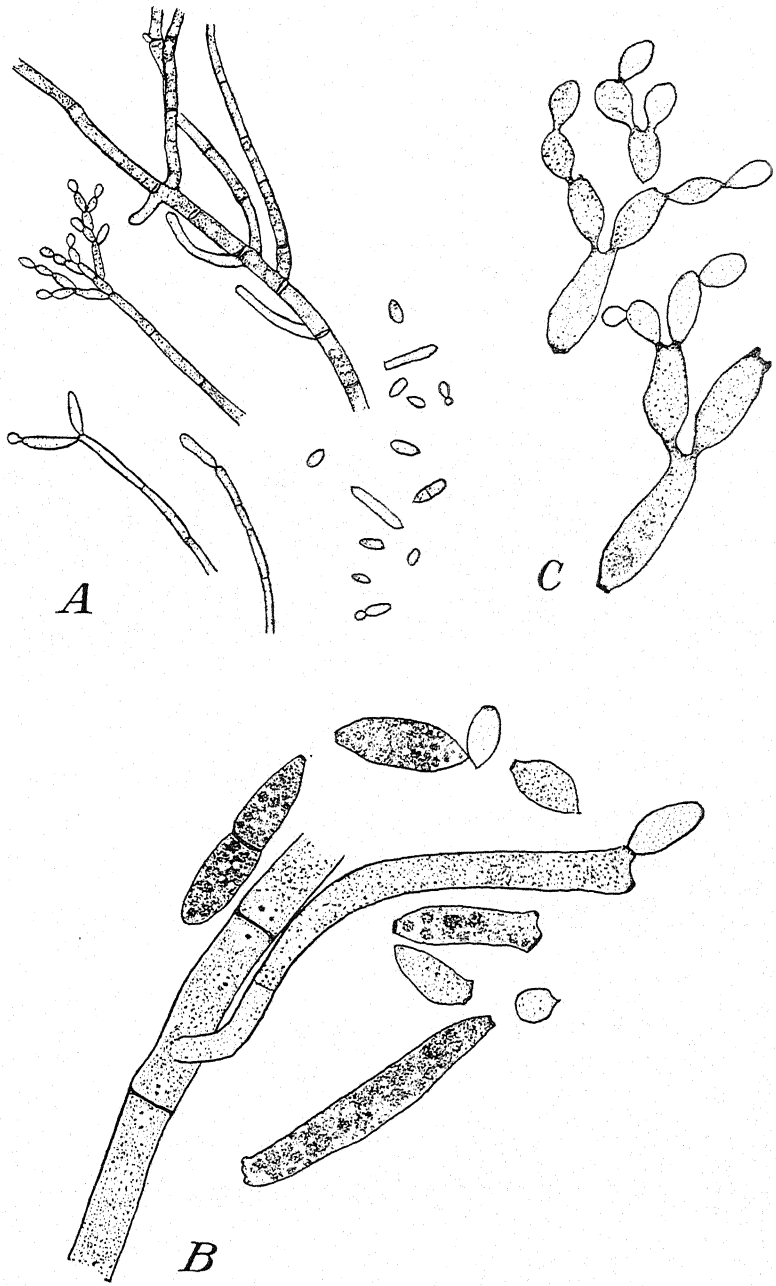


FIGURE 9.—Conidia and conidiophores of *Cladosporium herbarum* isolated from California figs. *A*, on Thaxter agar. ×450. Note the branching or arbor type of spore formation. *B*, On water agar. ×1,700. Showing details of conidial forms. *C*, On water agar. ×1,700. Part of a conidial chain showing dichotomous branching.

On figs and on agar cultures, the branching of the conidial arbor was predominantly dichotomous rather than sympodial, and the conidial formation was of the *Hormodendron* type. However, the comparative studies of Brooks and Hansford,[6] Bennett,[7] and Robak[8] place this *Hormodendron* as a form or variety of *Cladosporium herbarum* (Pers.) Link. The writers have compared the fungus under consideration with a culture of *Cladosporium herbarum* received from Dr. Johnanna Westerdijk, director of Centraalbureau voor Schimmelcultures, Baarn, Netherlands, and have found no significant difference between the two organisms. The fungus is therefore classified as *C. herbarum* (Pers.) Link.

While this *Cladosporium* has been isolated from figs and has produced spots when inoculated on the figs, it should be emphasized that it is not of great economic importance. Its occurrence has been confined largely to overripe fruit. The spotting of otherwise marketable figs is caused almost entirely by *Alternaria*.

BOTRYTIS

Botrytis sp. was occasionally found on overripe figs, but the fungus was inclined to spread over the surface of the fruit in a manner that set it entirely apart from the spotting under consideration.

MATERIALS AND METHODS

The fruit used in the present experiments was obtained from various sources, and the following lots will be referred to later by number. The variety names for the eastern lots are those given by the salesmen or the producers.

Lot 1.—Black Mission figs that had been held in storage at Exeter, Calif., for 15 days, July 1932, were the source of the original cultures of *Alternaria*.

Lot 2.—Kadota, Black Mission, Black Spanish, and Calimyrna figs were picked at Fresno, Calif., August 8, 1932, and forwarded to Yakima, Wash., by ordinary express. They arrived in Yakima August 10, free from mildew and in good condition, and were used in experiments the following day.

Lot 3.—Black Mission figs from Sultana, Calif., purchased on the Washington, D. C., market July 13, 1933.

Lot 4.—Black Spanish figs from Norfolk, Va., purchased on the Washington, D. C., market August 17, 1933.

Lot 5.—Calimyrna figs from California, purchased on the New York market August 25, 1933, and forwarded to Washington, D. C., by ordinary express.

Lot 6.—Brown Turkey figs from Norfolk, Va., purchased on the Washington, D. C., market September 6, 1933.

Lot 7.—Figs purchased from a local grower, Washington, D. C., September 11, 1933: (a) Black Spanish or related variety; (b) Green Ischia or related variety.

Lot 8.—Brown Turkey figs from Norfolk, Va., purchased on the Washington, D. C., market September 27, 1933.

Lot 9.—Black Mission figs from California, purchased on the Washington, D. C., market June 6, 1934.

Lot 10.—Black Mission figs from California, purchased on the New York market June 30, 1934, and taken to Washington, D. C., the same day.

Lot 11.—Calimyrna figs, purchased on the New York market August 9, 1934, and taken to Washington, D. C., the same day.

Lot 12.—Calimyrna figs, purchased on the New York market August 13, 1934, and forwarded to Washington, D. C., by ordinary express.

[6] BROOKS, F. T., and HANSFORD, C. G. MOULD GROWTHS UPON COLD-STORE MEAT. Brit. Mycol. Soc. Trans. 8: 113-142, illus. 1923.

[7] BENNETT, F. T. ON CLADOSPORIUM HERBARUM: THE QUESTION OF ITS PARASITISM, AND ITS RELATION TO "THINNING OUT" AND "DEAF EARS" IN WHEAT. Ann. Appl. Biol. 15: 191-213, illus. 1928.

[8] ROBAK, H. INVESTIGATIONS REGARDING FUNGI ON NORWEGIAN GROUND WOOD PULP AND FUNGAL INVASION AT WOOD PULP MILLS. Nyt. Mag. Naturvidenskab. 71: 185-330, illus. 1932.

Lot 13.—Calimyrna figs, purchased on the New York market August 20, 1934, and forwarded to Washington, D. C., by ordinary express.

Lot 14.—Calimyrna figs, purchased on the New York market August 17, 1935, and taken to Washington, D. C., the same day.

Lot 15.—Calimyrna figs, purchased on the New York market August 21, 1935, and forwarded to Washington, D. C., by ordinary express.

Lot 16.—Calimyrna figs, purchased on the New York market September 3, 1935, and forwarded to Washington, D. C., by ordinary express.

The inoculations were made in all cases by spraying the figs with sterile-water spore suspensions of organisms previously isolated from figs. After inoculation the figs were held in the open until dry before being placed under the experimental conditions. All of the figs were apparently free from spotting at the beginning of the experiments. In part of the tests, as mentioned later, the figs were disinfected with a bichloride of mercury wash prior to inoculation.

From 15 to 20 fruits were used under each condition in each experiment. Great care was used in selecting the figs so that those to be compared would be as nearly alike as possible in maturity and in freedom from injury.

Unless otherwise stated the spotting reported in the following experiments was of the type produced by *Alternaria tenuis*.

EFFECT OF HUMIDITY

Smith and Hansen[9] reported that fig spotting was likely to develop after summer rains or periods of high humidity and that at certain times during the season almost all figs of the white varieties would develop the disease if they were confined in a moist chamber.

TABLE 1.—*Effect of humidity on the development of spotting on figs*

Lot No.	Fungus	Temperature	Period of holding	Spots per fruit with—		
				High humidity [1]	Medium humidity [2]	Low humidity [3]
		° F.	Days	Number	Number	Number
3	*Alternaria*	59	4	31.8	5.8	
5	*Alternaria*	59	3	6.8	2.1	
8	*Alternaria*	59	3	1.6	.5	
9	*Alternaria*	50	2	1.9	1.1	
		40	3	1.3	.9	
		32	5	.8	.9	
10	*Alternaria*	50	2	1.6	1.4	
		40	2	.7	.3	
		32	4	3.4	2.2	
11	*Alternaria*	50	5	2.0	1.5	
		40	5	.6	.9	
		32	5	1.0	.8	
12	*Alternaria*	50	[4] 5	13.8	12.1	
		40	[4] 5	10.3	7.1	
		32	[4] 5	4.4	4.3	
13	*Alternaria*	50	[4] 5	3.1	2.9	
		40	[4] 5	1.4	1.1	
		32	[4] 5	1.9	1.8	
16	*Alternaria*	50	8	3.8	2.6	0
	Cladosporium	50	8	3.0	2.1	0

[1] Humidity of 90 percent or more.
[2] Humidity of 70 to 80 percent.
[3] Humidity of approximately 65 percent.
[4] During the last 3 of the 5 days all lots were at 50° F. and high humidity.

[9] SMITH, R. E., and HANSEN, H. N. See footnote 3.

In the present humidity studies the figs of lots 3, 5, and 8 were held in glass jars of 9-quart capacity and those of lot 16 and lots 9 to 13, inclusive, were held in metal boxes of approximately 1-cubic-yard capacity. Low humidity was maintained by the use of calcium chloride and high humidity by means of damp cloth or damp filter paper. With lot 3 the air was stagnant; with lots 5 and 8 it was slowly renewed; and with the remaining lots it was kept in motion by means of fans. The results of the experiments are shown in table 1.

All of the figs were inoculated, as previously described (p. 481.) The figs of lot 16 were disinfected with bichloride of mercury before inoculation; those of the other lots were not. An uninoculated lot at high humidity was included in most of the tests. The number of spots on the uninoculated figs was about one-fourth as great as that on the inoculated figs. The figs stored under medium humidity showed only slight shriveling, but it was evident in most cases that the moisture was as low as practicable for maintaining the product in a marketable condition. The figs held at low humidity (65 percent) soon became too badly withered to be marketable.

Storage at low humidity entirely prevented the development of spots. The figs held at medium humidity had less spotting in nearly every instance than those held at high humidity, but this reduction was seldom more than 30 percent. The effect of humidity appeared greater at 59° F. than at any of the lower temperatures. In the single test with *Cladosporium* the humidity effects were similar to those obtained with *Alternaria*.

The results show that humidity may be a factor of importance in the development of spotting, but they do not indicate that it is likely to form the basis for practical control.

EFFECT OF TEMPERATURE

The effect of temperature upon the development of fig spotting was tested both on fruit and on culture media.

Three series of experiments were made with the California *Alternaria* on culture media. In each series five Petri plates were used at each of the six temperatures. The curves of figure 10 give the average of the results of the different experiments

A study of the curves shows that the *Alternaria* growth rate was approximately twice as rapid at 77° as at 59° F., about twice as rapid at 68° as at 50°, nearly three times as rapid at 59° as at 41°, and fully three times as rapid at 41° as at 32°.

The effect of temperature upon the number of spots on the fruit is shown in table 2. In several of the tests the notes were taken after all of the lots had been held at a common temperature for 1 or more days after removal from the special temperatures. The results give a correct picture of what is likely to happen when figs are removed from storage, but do not show as great contrast as if the figs had been continued at the different storage temperatures until the time of note taking.

Notes taken at the time the figs were removed from the special storage conditions indicate that the effect of temperature on the number of *Alternaria* spots on the fruit is fully as great as the effect on the rate of growth in Petri dish cultures (fig. 10). In a single test with

the Virginia *Alternaria*, temperature contrasts were obtained similar to those reported in table 2 for the California *Alternaria*.

In the three tests with *Cladosporium* (table 2), the effect of temperature upon spotting was similar to that in the corresponding tests with *Alternaria*.

The spots at the higher temperatures were usually much larger than those at the lower temperatures as well as more numerous. There seems to be no doubt of the extreme value of low temperature in the prevention of spotting of figs.

EFFECT OF CARBON DIOXIDE

The effect of carbon dioxide upon *Alternaria* and *Cladosporium* was tested both on culture media and on the fruit. The storage atmos-

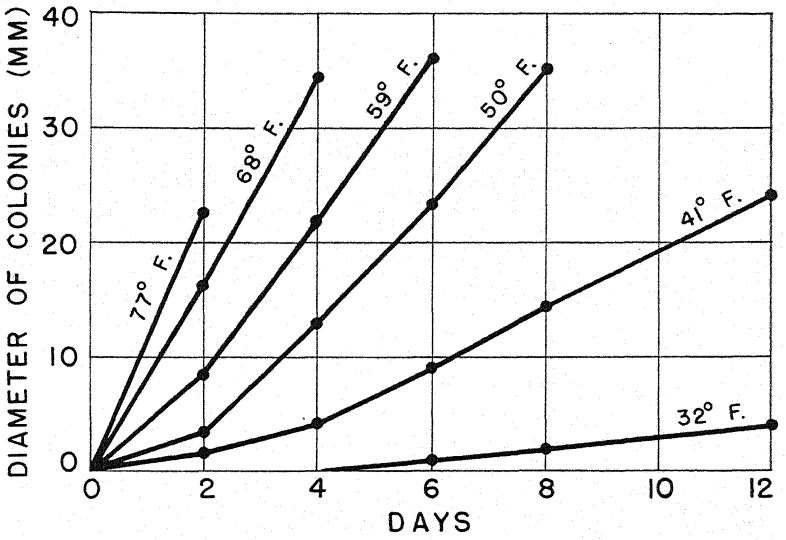


FIGURE 10.—Effect of temperature on growth rate of *Alternaria tenuis* cultures on potato-dextrose agar in Petri plates.

pheres were maintained by constant renewal with mixtures of carbon dioxide and ordinary air combined by means of flowmeters.

Two series of experiments were made in which *Alternaria* was grown on potato-dextrose agar in Petri plates. In each series five plates were used under each condition. In the series reported in figure 11 the carbon dioxide was maintained at approximately 30 percent, and in the series reported in figure 12 at approximately 50 percent. Similar lots of plates were held at the various temperatures in ordinary air. After 2 days' treatment with carbon dioxide the plates were removed from the prepared atmospheres and held with the control plates at the original temperature.

It was found in general that the 2 days' exposure to atmospheres containing 30 percent of carbon dioxide delayed the growth of the fungus about 1⅓ days and that a similar exposure to atmospheres containing 50 percent of carbon dioxide caused a delay of approximately 1½ days. The delay was fully as great at the lower as at the

TABLE 2.—*Effect of temperature upon the development of spots on figs that had been inoculated with a spore suspension of Alternaria or Cladosporium*

[Isolations from California figs]

Fungus and lot No.	Period of storage	Holding temperature after storage	Period held	Spots per fruit in lots removed from—				
				32° F.	41° F.	50° F.	59° F.	68° F.
Alternaria:	*Days*	° *F.*	*Days*	*Number*	*Number*	*Number*	*Number*	*Number*
3	4		0	1.7			31.8	
4	3	70	1	.3			3.5	
5	2	70	1	.8			6.8	
6	2	70	1	.1			.7	
8	2	70	1	.1			1.6	
9	2		0	.4	0.8	1.9		
10	2		0	.1	.7	1.6		
11	3	50	2	1.0	.6	2.0		
12	2	50	3	4.4	10.3	13.8		
13	2	50	3	1.8	1.4	3.8		
14	3		0	.0	.0	.3	.3	1.4
15	2	41	5	2.5	1.4	2.0	.9	6.6
16	5		0	.0	.0	.5	1.2	13.1
Cladosporium:								
14	3		0	.0	.1	.9	.7	1.8
15	2	41	5	1.6	2.6	2.9	8.7	12.6
16	5		0	.0	.0	.5	.7	4.7

higher temperatures. It is evident that during the period of treatment with carbon dioxide the activity of the fungus must have been reduced in the first case to roughly one-third of normal and in the second to about one-fourth of normal. At the higher temperatures this inhibition was slightly less than that resulting from a drop of 18° in temperature, as shown in figures 11 and 12.

The results from the carbon dioxide experiments with fruit are shown in table 3. Exposure at 59° or 68° F. to atmospheres containing 35 percent or more of carbon dioxide usually gave as great inhibition of spotting as holding at 32° without carbon dioxide. The results following *Alternaria* inoculations were somewhat more decisive than those following *Cladosporium* inoculations.

The figs of lot 7 (p. 480) were given carbon dioxide treatments similar to those reported in table 3, but the spotting was obscured by the development of *Rhizopus* sp. The carbon dioxide held this fungus in check as well as it held *Alternaria*.

In other experiments with fruit the carbon dioxide treatments were given in pony refrigerators similar to those used in the commercial shipment of Florida strawberries. The carbon dioxide gas was obtained by placing a few pounds of solid carbon dioxide in the refrigerator pan along with ordinary ice. With this method of treatment both the carbon dioxide and the temperature were lower at the end of the experiment than at the beginning. The conditions and the results of the experiments are shown in table 4.

In the six instances in which the carbon dioxide averaged 23 percent or more, the inhibition of spotting was as great as or greater than that resulting from immediate storage at 32° F. The effect was approximately equivalent to that of a drop of 20° in temperature. In the two instances in which the percentage of carbon dioxide was lower than 23 percent, the spotting was worse than that obtained by immediate storage at 32°, but decidedly less than that obtained in the control refrigerators without carbon dioxide.

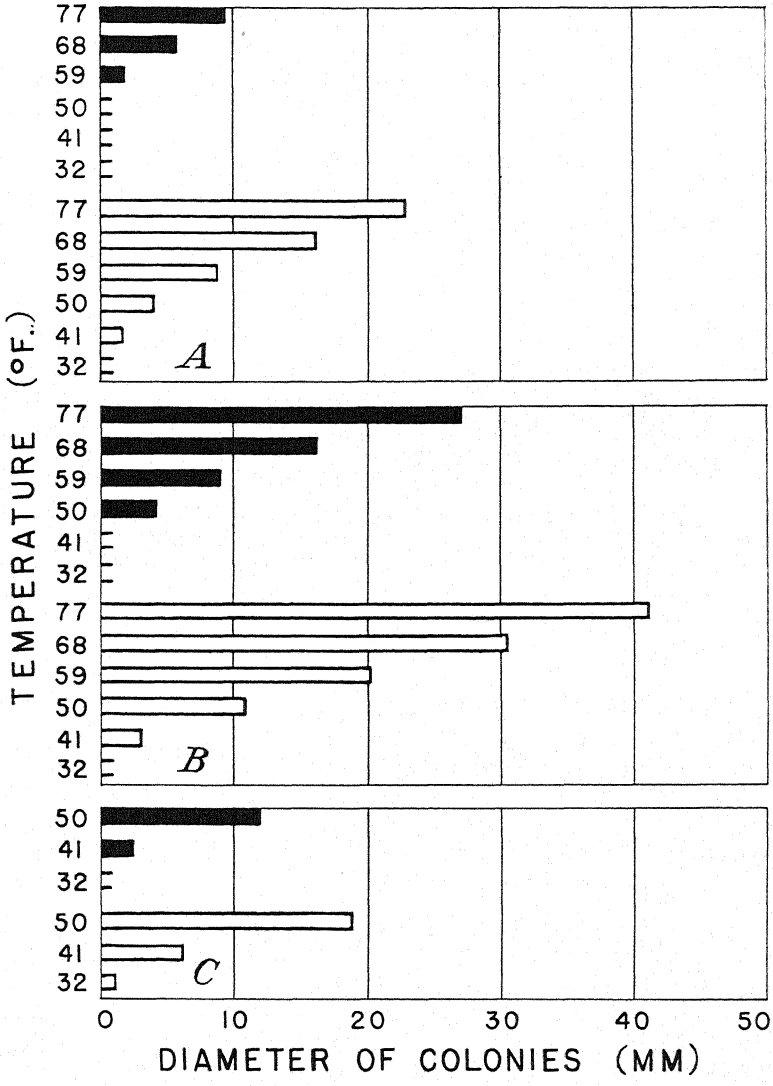


FIGURE 11.—Effect of carbon dioxide at various temperatures on growth rate of cultures of *Alternaria tenuis* on potato-dextrose agar in Petri plates. After 2 days' storage in air containing about 30 percent CO_2, the treated lots were placed with corresponding control lots and continued at the original storage temperatures. Graphs show diameters of colonies at end of (*A*) 48 hours, (*B*) 80 hours, and (*C*) 120 hours. Solid bars, lots treated with CO_2 for first 48 hours; open bars, lots stored without CO_2.

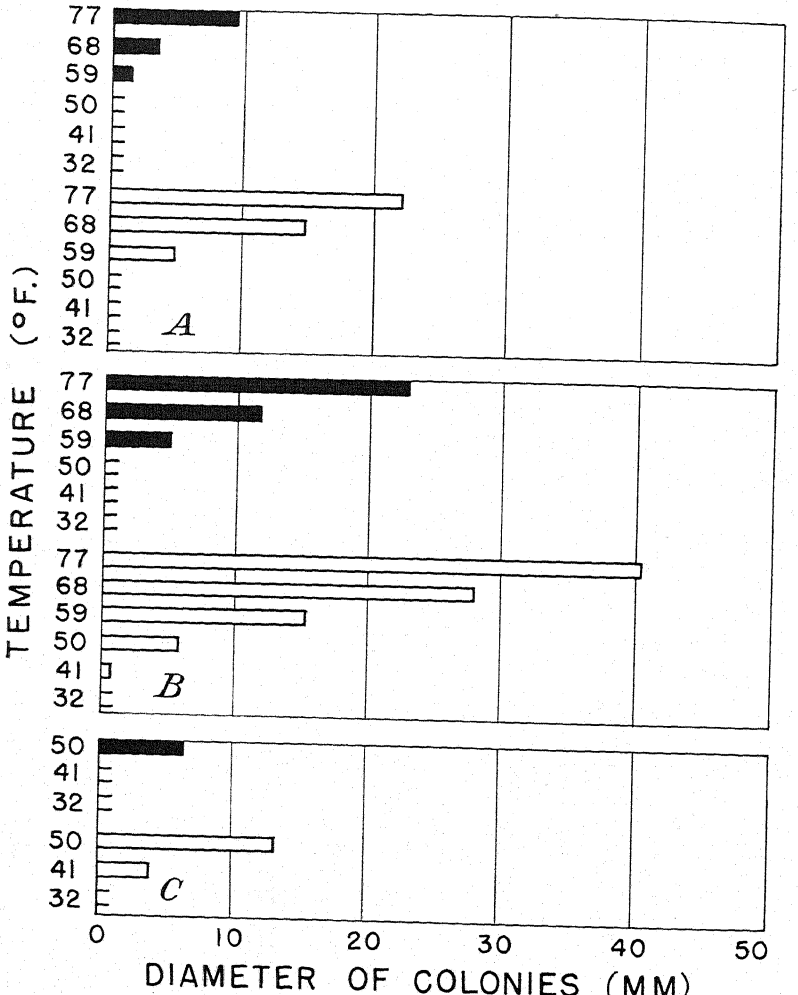


FIGURE 12.—Effect of carbon dioxide at various temperatures on growth rate of cultures of *Alternaria enuist* on potato-dextrose agar in Petri plates. After 2 days' storage in air containing about 50 percent CO_2, treated lots were placed with corresponding control lots and continued at the original storage temperatures. Graphs show diameters of colonies at end of (*A*) 48 hours, (*B*) 84 hours, and (*C*) 120 hours. Solid bars, lots treated with CO_2 for first 48 hours; open bars, lots stored without CO_2.

TABLE 3.—*Effect of carbon dioxide upon the spotting of figs inoculated with Alternaria or Cladosporium*

Fungus and lot No.	Experimental period	Temperature during experimental period	Carbon dioxide during experimental period	Period held after treatment	Holding temperature after treatment	Spots per fruit
Alternaria:	*Days*	° *F.*	*Percent*	*Days*	° *F.*	*Number*
		59	50	0	----------	0.1
3	4	59	35	0	----------	1.8
		59	0	0	----------	31.8
		32	0	0	----------	1.7
		59	50	1	70	.6
4	3	59	0	1	70	4.2
		32	0	1	70	.3
		59	50	1	70	.8
5	2	59	0	1	70	6.8
		32	0	1	70	.8
		59	50	1	70	.1
6	2	59	0	1	70	.7
		32	0	1	70	.1
		59	50	1	59	.0
8	2	59	0	1	59	1.6
		32	0	1	59	.1
		68	35	0	----------	.0
		68	0	0	----------	1.3
		32	0	0	----------	.0
		41	0	0	----------	.0
		50	0	0	----------	.3
14	3	59	35	1	50	.0
		59	0	1	50	.5
		32	0	1	50	.2
		41	0	1	50	.2
		50	0	1	50	.6
		68	35	5	41	1.5
		68	0	5	41	6.6
		59	35	5	41	.9
15	2	59	0	5	41	4.7
		41	0	5	41	1.4
		50	0	5	41	2.0
Cladosporium:						
		68	35	0	----------	.0
		68	0	0	----------	1.2
		32	0	0	----------	.0
		41	0	0	----------	.1
		50	0	0	----------	.9
14	3	59	35	1	50	.0
		59	0	1	50	2.0
		32	0	1	50	.1
		41	0	1	50	.2
		50	0	1	50	.4
		68	35	5	41	2.1
		68	0	5	41	12.6
		59	35	5	41	2.9
15	2	59	0	5	41	8.7
		32	0	5	41	1.6
		41	0	5	41	2.6
		50	0	5	41	2.9

TABLE 4.—*Effect of carbon dioxide upon the spotting of figs* [1]

Lot No.	Period of treatment	CO_2 in atmosphere during treatment			Temperature during period of treatment			Period held after treatment	Holding temperature after treatment	Spots per fig
		Early high	Final low	Average	Initial high	Final [2]	Average			
	Days	*Percent*	*Percent*	*Percent*	*° F.*	*° F.*	*° F.*	*Days*	*° F.*	*Number*
10	2	55	30	39	69	59	61	0	--------	0.0
10	2	34	14	22	69	53	56	0	--------	.3
10 [3]	2	--------	--------	--------	69	47	51	0	--------	.9
10 [4]	2	--------	--------	--------	32	32	32	0	--------	.1
10 [4]	2	--------	--------	--------	41	41	41	0	--------	.7
11	3	64	25	40	75	49	52	0	--------	.6
11	3	46	3	26	75	49	49	0	--------	.7
11 [3]	3	--------	--------	--------	75	48	47	0	--------	2.5
11 [4]	3	--------	--------	--------	32	32	32	0	--------	1.0
12	2	47	30	38	72	47	50	3	50	3.1
12	2	38	11	23	72	42	46	3	50	3.6
12 [3]	2	--------	--------	--------	72	44	47	3	50	9.1
12 [4]	2	--------	--------	--------	32	32	32	3	50	4.4
12 [4]	2	--------	--------	--------	41	41	41	3	50	10.3
13	2	64	33	44	54	47	46	3	50	1.9
13	2	27	2	12	54	44	44	3	50	3.2
13 [3]	2	--------	--------	--------	54	42	42	3	50	6.4
13 [4]	2	--------	--------	--------	32	32	32	3	50	1.9

[1] The carbon dioxide treatments were given in pony refrigerators. The figs were sprayed with an *Alternaria* spore suspension several hours before the experiments were started.
[2] The final temperature was not always the lowest.
[3] Control; not treated with CO_2.
[4] Placed immediately in low-temperature storage.

The figs were carefully tested as to flavor after the various carbon dioxide treatments. No difference could be detected between the treated and the untreated fruit.

It is evident that in the absence of satisfactory precooling facilities, valuable protection can be obtained by the proper use of solid carbon dioxide.

SUMMARY

The spotting of figs on the market is found to be due mainly to *Alternaria tenuis* Nees, but *Cladosporium herbarum* (Pers.) Link is also capable of producing spots.

Experiments are reported on the effects of humidity, temperature, and carbon dioxide upon the development of spotting.

Spotting was decreased by lowering the humidity of the storage atmosphere, but humidities that gave satisfactory control also caused severe shriveling.

The growth rate of the *Alternaria* on potato-dextrose agar was approximately twice as great at 77° as at 59° F., nearly three times as great at 59° as at 41°, and fully three times as great at 41° as at 32°.

At temperatures of 41°, 50°, 59°, 68°, and 77° F., exposure to 30 percent of carbon dioxide reduced the activity of the *Alternaria* to about one-third of normal.

With temperature conditions somewhat less satisfactory than those usually found during the first 2 days in a nonprecooled car, exposure to atmospheres in which the carbon dioxide averaged 23 percent or more gave as good control of spotting as immediate storage at 32° F.

EFFECT OF LOW CONCENTRATIONS OF COPPER ON GERMINATION AND GROWTH OF CONIDIA OF SCLEROTINIA FRUCTICOLA AND GLOMERELLA CINGULATA [1]

By M. C. GOLDSWORTHY, *associate pathologist*, and E. L. GREEN, *associate biochemist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Spores of a fungus attached to a surface that has been sprayed with a copper-containing fungicide such as bordeaux mixture are in contact with a residue which, when wet, becomes a dynamic physical and chemical system. Although the quantity of soluble copper and the concentration of ions in this system fluctuate with changing conditions, its toxicity to fungus spores appears to be correlated with the available copper originally in the residues or later built up in them, as Goldsworthy and Green (*7*)[2] have shown. It has been demonstrated by Ribéreau-Gayon (*13*), Hockenyos (*9*), McCallan and Wilcoxon (*11*), and Goldsworthy and Green (*7*) that the clear supernatant liquid of freshly prepared bordeaux mixture spray contains several parts of copper per million. McCallan and Wilcoxon (*11*) have shown that, when bordeaux preparations are dried on glass surfaces and rewet, the soluble copper amounts to 0.2 to 0.3 p. p. m.

The data presented by Goldsworthy and Green (*7*) appear to demonstrate that it is the available [3] copper in the dried but rewet bordeaux mixture residues that is responsible for the toxicity of the residues to the conidia of *Sclerotinia fructicola*. McCallan and Wilcoxon (*11*), on the other hand, concluded that the small solubility of the copper in these residues is not sufficient to cause toxicity. They state that "this concentration of copper is insufficient to affect materially the germination of spores of the species of fungi used." This statement may be true of a system in which the concentration of copper is immediately lowered by absorption and not replaced, but it appears logical to assume that in a dynamic system, such as that which is presented by a drop of water in contact with a residue of bordeaux mixture, copper ions absorbed by the spores would be immediately replaced from the residue and the available copper would be sufficient to cause toxic effects. This explanation of such a system is in accordance with the oligodynamic theory of Nägeli (*12*).

The present paper reports the results of experiments on the toxicity of low concentrations of ionized copper, in copper sulphate solutions and in saturated solutions of spray materials containing relatively insoluble copper, to the conidia of *Sclerotinia fructicola* (Wint.) Rehm and *Glomerella cingulata* (Ston.) Spauld. and Schrenk. In conjunction with these experiments, studies were also made on the toxicity of ionized copper when added to various substrates and forming a system considered as static since the original concentration of copper ions was not continuously maintained.

[1] Received for publication August 30, 1937; issued May 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 504.

[3] The "available" copper is defined as that copper which may be readily absorbed by the conidia, with toxic effects, and which may or may not be ionic in nature.

MATERIALS AND METHODS

The conidia used in these experiments were from artificial cultures grown at room temperature and of an age previously determined to be that at which the highest percentage of the conidia were viable. For *Sclerotinia fructicola* growing on potato hard agar and on string beans, this age was 14 days and 7 days, respectively; for *Glomerella cingulata* growing on potato hard agar, it was 4 days. The conidia of *S. fructicola*, after being freed from the surface of the various media either by washing or by suction, were placed in a small amount of distilled water in which they were made to stay in suspension by the addition of a minute quantity of the preparation described by Allison, Hoover, and Burk (*1*) as coenzyme R. The conidia of *G. cingulata* were washed free from their conidiophores with distilled water. The conidia of both species were then seeded in water agar and were distributed so that under the lowest power of the microscope 8 to 12 conidia were in the field at one time. Small blocks of the conidia-containing water agar, 1 by 4 by 4 mm, were then placed in the perfusion apparatus described in the next paragraph, or on agar containing different nutrients and various amounts of copper.

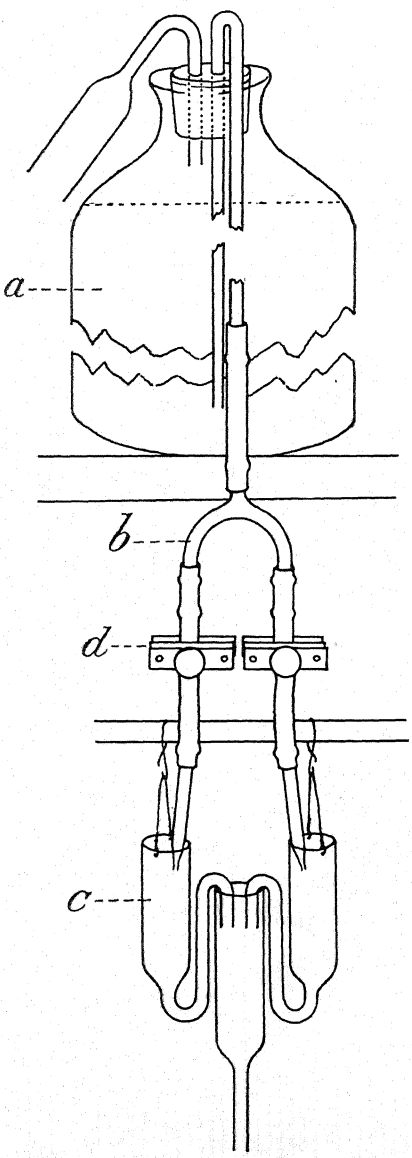


FIGURE 1.—Perfusion apparatus: *a*, Test solution; *b*, siphon system; *c*, perfusion cells; *d*, screw clamps.

The perfusion apparatus (fig. 1) consisted of a supply bottle, containing 2 liters of test solution (*a*), from which the solution was delivered by gravity through a siphon system (*b*) to the perfusion cells (*c*). The flow into the perfusion cells was regulated by screw clamps (*d*), so that the 2 liters of solution in the supply bottle passed over the conidia-containing water-agar cubes during a 24-hour period. The perfusion cells in which the water-agar blocks were placed were built so as to maintain a constant level of liquid by an overflow siphon while allowing a charge of conidia-containing units to be continuously washed with the solution to be tested. In this manner the conidia were subjected to a constant concentration of soluble and

ionized copper, and the amount of copper absorbed could be deduced by determining the total copper remaining in the overflow from the perfusion cells.

The system just described is dynamic and closely simulates the system in operation on a sprayed plant where, under weather conditions favorable to infection, conidia are suspended in a drop of rain on the surface of a film of residue. The drop of rain becomes saturated with the soluble constituents of the residue and remains so until dried up by a change in environmental conditions. If the drop of rain remains long enough the conidium begins activity and then absorbs copper, which is replaced from the residue. The system operates until the conidium is killed or inhibited, provided the store of available copper in the residue is not too small. Such a system is probably not static, because the small amount of copper dissolved in the raindrops on these residues is being continuously replaced after it is absorbed by conidia.

The perfusion apparatus afforded an opportunity to study the reactions of conidia immersed in a saturated solution of a fungicide but separated from contact with the undissolved particles. The rate at which this solution was renewed was such that absorption of copper by the conidia could not significantly change the concentration.

In many of these tests the copper-containing solution was the primary saturated solution of various relatively insoluble copper materials, obtained by shaking intermittently for 48 hours 20 grams of each material with 2 liters of distilled water. The primary solution is defined here as the solution obtained from the first washing; it probably contains, in addition to the soluble portion of the copper material, slight amounts of other soluble ions that were present when the copper material was precipitated out of solution. In addition to these, especially with the commercial compounds used in the experiments, relatively large amounts of various kinds of wetting or stabilizing agents may also be present in the primary solution. After the undissolved residue of these solutions had settled out, which usually required several days, the liquid portion was filtered off by gravity or suction through a Chamberland-Pasteur filter cylinder having an L2 porosity. The filtrate obtained in this manner was then used in the perfusion apparatus. Similar filtrates were tested for their copper content, and in many cases the perfused filtrates were collected and tested for copper.

Since the solutions were clear, no particular caution was necessary in securing samples for analysis. To improve the accuracy of the analysis, it seemed desirable to take a relatively large sample and evaporate it to small volume after adding about 5 ml of nitric acid to make sure that the copper compound present did not come out of solution.

With certain of the organic copper compounds the deep color of the concentrated solution showed plainly that the amount of copper was far beyond the range of the micromethod. These samples were transferred to evaporating dishes, evaporated to dryness, and ignited below red heat in the electric muffle furnace. After cooling, the ash was dissolved in dilute nitric acid and transferred to volumetric flasks, from which portions were taken for analysis by the iodometric method recommended for the determination of copper in fungicides by the Association of Official Agricultural Chemists (*3*).

Haas and Quayle (*8*) have proposed a method for the determination of small quantities of copper that is essentially a small-scale modification of the method mentioned above. The thiosulphate solution is diluted to 0.002 normal from 0.10 normal, but the other steps in the procedure are followed exactly. In this method the cuprous iodide formed remains in solution, yet there seems to be no difficulty in determining the end point. From results with this method it was thought that the determination of intermediate quantities of copper could be made by using intermediate dilutions of the thiosulphate. This was found to be true, and for such quantities of copper this method was employed. The titer of the thiosulphate solution was determined on the day of use by including electrolytically standardized solutions of copper sulphate along with the samples to be analyzed.

The procedures described are scarcely suitable for the determination of less than 0.2 mg (200 micrograms) of copper, because the titer of a 0.002-normal solution of sodium thiosulphate is about 140 micrograms of copper per million. Of the procedures tried, the method of Callan and Henderson (*4*), as modified by Delage (*5*), was found the most convenient for quantities of copper between 10 and 200 micrograms. No attempt was made to determine smaller amounts, because the necessary special precautions, such as the use of purified reagents and purified water, were not taken.

During the period of operation of the perfusion apparatus, the test conidia were examined from time to time. The small cubes of conidia-containing water agar were fished out of the perfusion cell and tested for germination either under the microscope or on nutrient media. Since the perfusion apparatus does not entirely exclude contaminating organisms, resistant contaminants occasionally survived the treatments and grew after transfer to culture media.

In addition to the dynamic system just described, a static system was used which consisted of placing the small cubes of conidia-containing water agar on the surface of water-agar, Czapek's-agar, and potato-agar slants to which dilute solutions of copper sulphate had been added. The copper solutions added to these media were diluted by adsorption by the agar, fixation by the various nutrients, and absorption by the conidia. Copper ions react specifically with alcohol hydroxyl groups, and in every one of the above media, even the plain water agar, almost every constituent has such groups. In these systems, therefore, little was known concerning the active concentration of copper. After a 24-hour period of contact with the copper-containing medium, the conidia-containing blocks were transferred to copper-free nutrient media to test the germinability of the conidia.

EXPERIMENTS WITH THE PERFUSION APPARATUS

EFFECT OF DISTILLED WATER

Distilled water is not usually a good medium in which to germinate the spores of fungi. Why this is true is not clear, since it is agreed by botanists that the spore of a fungus usually contains the means to initiate growth. It would seem logical to assume, provided plasmoptysis does not take place, that the presence of pure water at the proper temperature would be sufficient to cause a swelling of the protoplasm, an activation of the enzymes, and resultant growth. But

this is not always so, as Duggar (*6*) has pointed out, and even among the spores of the same organism there is great variability in the response to distilled water.

Germination in distilled water can be greatly invigorated, however, as Duggar (*6*) and Wilcoxon and McCallan (*15*) have shown, by the addition of some accessory substance, such as a trace of malic acid or some plant decoction. These responses indicate strongly that the spores are dependent on some outside source of energy or stimulation before growth can begin.

Smart (*14*) indicated that a supplementary or external supply of food was necessary to the proper germination of the spores of a number of Myxomycetes. He further showed that single spores do not germinate, but that masses of spores favor germination and that single spores placed in an environment previously inhabited by germinating spores will germinate freely. These facts surely indicate that the spores of species of Myxomycetes are dependent upon some influence other than that furnished by the water for the stimulation of germination. This "autocatalytic" agent probably may be better regarded as some particular nutritional substance derived from the external food supply.

When spores are formed in contact with a food-containing medium, it is possible that all of them, on becoming detached, carry a supply of food or growth-promoting materials on their surfaces. The varying degrees of germination so often observed in distilled water may be due to the varying quantities of these materials carried by the individual conidia. That the conidia of various species have on their surfaces different amounts of materials that stimulate growth is indicated by the work of McCallan and Wilcoxon (*11*). These investigators also show that hydroxy acids are present on conidia derived from nutrient media and that the same or similar materials may be isolated from the surface of used and sterile nutrient agars. While they assume that the hydroxy acids (e. g., malic acid) and other materials isolated from conidia are secreted by conidia, they have not demonstrated that these materials are not also carried over from the medium in which the conidia are grown. Their washing experiments, which show that the assumed secretion may be almost entirely removed in the primary washing of the conidia, appear to indicate that these materials may have been taken up from the medium on which they had grown or that, if secreted by the organism, they are secreted slowly and therefore are not readily replaced. The findings of McCallan and Wilcoxon in general indicate that conidia from a nutrient substrate have adsorbed on their surfaces nutritive materials that may help to initiate germination.

In the perfusion tests described in this paper in which distilled water was the perfusion solution and water agar the suspension medium, germination of the conidia of *Glomerella cingulata* was usually only 6 to 36 percent and that of *Sclerotinia fructicola* rarely more than 5 percent. In all cases the germ tubes were subnormal, both in length and in vigor.

Experiments of various types were conducted to find out why the conidia of both species failed to germinate to their full capacity under these conditions. When the perfused conidia of both species were transferred to water-agar surfaces that had been freed as nearly as possible of all traces of nutrient materials by dialysis, only a slight

increase in germination occurred; but when the transfer was made to a nutrient medium, such as potato agar, germination was more rapid and more vigorous than normal and the percentage of germination was higher. It appears, therefore, that both species are dependent upon certain materials to stimulate germination, and that these materials are removed when the conidia are subjected to a period of washing and are restored when the conidia are transferred to a nutrient medium.

These conidia also appeared to have been activated by the perfusion treatment to a point where the addition of stimulatory substances caused immediate growth. It is not clear why the conidia of *Glomerella cingulata* germinated more freely during the perfusion process than did those of *Sclerotinia fructicola*. That conidia of *S. fructicola* collected by suction are reasonably free of soluble materials from the culture media may account for the lower percentage of initial germination always observed with this species. On the other hand, conidia of *G. cingulata* cannot be collected cleanly from the surface of the culture medium by suction, but generally must be removed by washing. These washed conidia carry over to the water-agar block a varying quantity of soluble nutrient materials that may account for the differences in germination observed between the two species.

In the perfusion tests, the percentage of germination of *Sclerotinia fructicola* conidia collected from string bean culture surfaces by suction was always lower than that of the conidia collected by washing. If the conidia of both *S. fructicola* and *Glomerella cingulata* are suspended in potato or potato-dextrose-agar blocks, which of course contain food materials, instead of water-agar blocks, which contain practically none, and are subjected to perfusion with distilled water, most of the conidia germinate and put out robust germ tubes in a few hours. As the washing continues, with consequent removal of the soluble food and accessory materials, the rate of growth of the germ tube diminishes and its final increment of growth becomes very thin as though the conidium had been starved. When these germinated conidia are placed in contact with a ready supply of soluble nutrients, they begin to grow again and to show the characteristics associated with sufficient nourishment. These experiments indicate that the initial growth in water- and nutrient-agar blocks is made at the expense of some soluble accessory substance which is removed by the perfusion operation.

The perfused conidia appeared not to be injured or changed in any manner by the distilled-water treatment, since it was always found that when perfused conidia were transferred to a suitable medium the percentage of germination was as high as that of unperfused conidia. Although the perfused conidia seem to be arrested in their development by the distilled-water treatment, they appear, as shown by the following experiments, to be able to absorb mineral constituents added to the perfusion liquid.

EFFECT OF LOW CONCENTRATIONS OF IONIZED COPPER

In the determination of the effect of low concentrations of ionized copper on the conidia of *Sclerotinia fructicola* and *Glomerella cingulata* in the perfusion apparatus, copper sulphate in distilled water, at copper concentrations of 4.0, 3.0, 2.0, 1.0, 0.5, and 0.25 p. p. m. was used. A number of experiments were conducted, and the results

of most of them agree with those reported in tables 1 and 2. The slight differences between the data in table 1 and table 2 are accounted for by the variations in the final germinability of the check conidia of *S. fructicola*. The toxicity of the higher concentrations was so quickly apparent that by the time activity was noticeable in the check conidia plasmolysis had occurred in the treated conidia. Since with the lowest dilutions (0.50 and 0.25 p. p. m.) the toxic effects developed more slowly, a more detailed study of the progress of the reaction was made. The data presented in table 2 are characteristic of the manner in which the conidia of both species behave when subjected to very low concentrations of copper ions. The absorption of copper was delayed, possibly by the lack of activity on the part of the organism or possibly by the initial absorption or inactivation of copper by the agar, but as the experiment progressed most of the conidia of both species were killed. In most cases a few conidia survived the treatments, even when the solutions had a high concentration. Apparently these conidia remained dormant or their activity was so slight that they absorbed sublethal quantities of copper.

TABLE 1.—*Toxic effect of low concentrations of copper sulphate solutions on the conidia*[1] *of Sclerotinia fructicola and Glomerella cingulata after a 24-hour perfusion period*[2]

Species	Copper concentration	Condition of conidia at end of perfusion period	Subsequent germination	
			Treated conidia	Check conidia[3]
	P. p. m.		*Percent*	*Percent*
S. fructicola	4.0	Nearly all plasmolyzed	6	98
	3.0	None have germinated; many distinctly plasmolyzed; a few appear turgid and normal.	4	96
	2.0	None have germinated; none appear turgid; all plasmolyzed.	8	98
	.50	None have germinated; all appear plasmolyzed	6	96
	.25	Some appear not injured; many appear plasmolyzed; a few have germinated.	16	94
G. cingulata	4.0	All appear plasmolyzed	0	100
	3.0	do	0	98
	2.0	do	0	100
	1.0	Nearly all cells appear plasmolyzed; an occasional cell appears turgid.	[4] 2	100
	.50	About 8 percent have germinated, but all appear plasmolyzed.	[4] 4	98
	.25	About 10 percent have germinated; most appear plasmolyzed; a few appear turgid.	[4] 2	100

[1] *S. fructicola* conidia were collected from 7-day-old cultures growing on string bean suspended in distilled water plus a small amount of coenzyme R; *G. cingulata* conidia were collected from 4-day-old potato-agar cultures by being washed off with distilled water.
[2] Period allowed for passage of solution over conidia suspended in water-agar blocks.
[3] Check conidia, also suspended in water-agar blocks, were perfused with distilled water.
[4] Continued activity.

The results obtained show that both species are very sensitive to low but constant concentrations of copper ions, once growth activity has begun and an equilibrium has been reached between the copper solution and the agar. Since spraying tests in the field indicate that *Glomerella cingulata* is more resistant to copper than *Sclerotinia fructicola*, it is generally supposed that the conidia of *G. cingulata* are more resistant to copper. Under the conditions of these experiments, when the conidia are deprived of their adherent supplements, it may be concluded that both species are equally susceptible to ionized copper. Under natural conditions, conidia of *G. cingulata* are usually

covered with a mucilaginous material that may bring about apparent resistance to copper sprays by the fixing or inactivation of ionized copper.

TABLE 2.—*Progressive toxic effect of low concentrations of copper sulphate solutions on the conidia of Sclerotinia fructicola and Glomerella cingulata* [1]

Original concentration of copper	Concentration of copper at end of perfusion period	Perfusion period [2]	Subsequent germination of—			
			Treated conidia		Check conidia [3]	
			S. fructicola	*G. cingulata*	*S. fructicola*	*G. cingulata*
P. p. m.	*P. p. m.*	*Hours*	*Percent*	*Percent*	*Percent*	*Percent*
0.25	----------	1.0	82	98	88	98
.25	----------	2.0	56	78	88	98
.25	----------	4.5	48	66	84	98
.25	0.21	24.0	16	8	86	98
.50	----------	1.0	74	98	88	98
.50	----------	2.0	48	82	88	98
.50	----------	4.5	33	46	84	98
.50	.48	24.0	14	6	86	98

[1] *S. fructicola* conidia were collected from 7-day-old cultures growing on sterile bean; *G. cingulata* conidia were collected from 7-day-old potato-agar cultures.
[2] Period allowed for passage of solutions over the conidia suspended in water-agar blocks.
[3] Check conidia, also suspended in water-agar blocks, were perfused with distilled water for 24 hours.

In a few experiments with copper concentrations of 0.25 and 0.50 p. p. m., the perfused filtrates were collected and analyzed for residual copper. It was usually found that only a small proportion of the original copper was absorbed by the conidia (table 2, column 2). Staining the killed conidia with the copper-sensitive chromotropic acid (*2*) indicator demonstrated that a quantity of copper sufficient to furnish a distinct color reaction had been absorbed and fixed by them. Since in another part of this paper the authors show that agar also has the property of absorbing copper, it appears that the loss of copper during perfusion is accounted for by absorption on the part of both conidia and agar. These experiments indicate that only a very small amount of ionic copper was necessary to inactivate a great number of conidia, and it seems logical to believe that there is sufficient available copper associated with bordeaux mixture residues to be toxic.

EFFECT OF SATURATED SOLUTIONS OF VARIOUS COPPER COMPOUNDS

Saturated solutions of various copper compounds were obtained by shaking intermittently by hand, at room temperature, over a period of 48 hours, 20 g of the copper materials with 2 liters of distilled water. After the undissolved material had settled out, the supernatant liquid was filtered through an L2 Chamberland-Pasteur filter candle either by gravity or by suction. The clear primary solution thus obtained was then employed in the perfusion tests and also in the analytical determinations. However, it was found that the perfusion tests could be conducted with the supernatant fluid without filtering, since the agar of the water-agar blocks apparently prevents the small amount of the solid phase which is unavoidably present from coming in contact with the suspended conidia. The perfusion tests were conducted in the same manner as those described previously. The results are shown in table 3.

TABLE 3.—*Toxic effect of primary saturated solutions obtained from various copper compounds on the conidia of Sclerotinia fructicola and Glomerella cingulata* [1]

Copper compound	Subsequent growth of test conidia after being treated with primary perfusion solution for—								Solubility of primary saturated solution
	2 hours		4 hours		6 hours		24 hours		
	S. fructicola	*G. cingulata*	*S. fructicola*	*G. cingulata*	*S. fructicola*	*G. cingulata*	*S. fructicola*	*G. cingulata*	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*P.p.m.*
Phosphate	100	100	100	100	100	100	98	100	0.17
Silicate	100	100	96	98	96	100	92	100	.48
Ammonium silicate	100	100	100	100	100	100	100	100	.20
Oxide (cupric)	100	100	100	100	98	100	100	100	.11
Oxide (cuprous)	45	42	12	Trace	4	0	0	0	26.7
Oxychloride:									
1	15	18	12	8	8	4	0	0	160.0
2	100	100	92	98	78	100	8	100	8.0
Basic sulphate	91	86	82	72	46	21	0	1	11.5
Zeolite:									
1	100	100	100	100	100	100	98	100	.05
2	100	100	100	100	100	100	96	100	.23
Malate	100	82	100	78	100	81	68	12	1,240.0
Maleate	86	0	46	0	21	0	Trace	0	315.0
Basic maleate	92	0	93	0	25	0	Trace	0	494.0
Resinate	100	100	97	100	96	100	100	100	.086
Oxalate	100	100	100	100	100	100	31	100	4.3
Check [2]							100	100	

[1] *S. fructicola* conidia collected from 7-day-old bean culture; *G. cingulata* conidia, from 4-day-old potato-agar culture.

[2] Check conidia were perfused with distilled water for 24 hours.

The copper compounds tested were, for the most part, commercial materials that showed some promise of becoming useful as fungicides. Of the inorganic materials, black copper oxide, copper silicate, copper ammonium silicate, copper phosphate, and copper zeolites were found to be nearly insoluble, and the copper in solution appeared to be either in an unavailable combination or available in quantities so minute that toxicity was negligible. Most of these materials, when tested in field experiments against the apple scab organism (*Venturia inaequalis* (Cke.) Winter) have not proved sufficiently useful to replace lime-sulphur solution during the seasons in which the disease is severe. They are useful, however, when mild fungicides that are relatively noninjurious to apple trees may be safely used.

The primary saturated solutions of red copper oxide, basic copper sulphate, and one of the copper oxychlorides (1) were consistently toxic to the conidia of both *Sclerotinia fructicola* and *Glomerella cingulata*, and the degree of toxicity appeared to be directly correlated with the amount of soluble copper present. Another one of the copper oxychlorides (2), despite its relatively high solubility, was found to be only slightly toxic to the conidia of *S. fructicola* and nontoxic to the conidia of *G. cingulata*. Why this particular copper oxychloride should show such a low degree of toxicity is not known. The copper solubilities of the two copper oxychloride materials are different, but, according to the authors' findings with ionic copper, the quantity of soluble copper should be sufficient in both cases to be toxic to the conidia of both fungi.

Orchard tests in various parts of the country[4] indicate that the black copper oxide, copper phosphate, copper ammonium silicate, copper zeolites, and copper silicate are not so effective against certain parasitic fungi as the more soluble red copper oxide, basic copper sul-

[4] Unpublished data.

phates, and certain copper oxychlorides. It is certain that red copper oxide, basic copper sulphate, copper ammonium silicate, and copper oxychlorides when used with lime are at times very injurious to the fruit and leaves of certain species of higher plants, whereas black copper oxide, copper phosphate, copper zeolites, and copper silicate are not.

Of the organic materials used, copper resinate, copper oxalate, and copper malate proved to be the least effective, whereas copper maleate and basic copper maleate were very toxic. It appears possible that in these compounds the copper is principally combined in complex molecules, since its solubility varies a great deal among the compounds and since their toxicity is not correlated with their degree of solubility. The solubility of the malate is of a high order, but a saturated solution of this compound is not quite so toxic as a dilute solution of copper sulphate containing a copper concentration of 0.25 p. p. m. The organic copper compounds also bring into solution large quantities of acid radicals that may affect toxicity. The toxic effects of the malate are not constant, possibly because of the enormous amount of malic acid combined with the soluble copper. In the case of copper oxalate, the effect of the acid appears to be even more marked. A saturated solution of copper oxalate was apparently nontoxic to conidia of *Glomerella cingulata* and was only slightly injurious to conidia of *Sclerotinia fructicola*. Nine parts per million of oxalic acid, which is the equivalent of that present in the copper oxalate solution used, appears to exert the same differential effect on the conidia of the two species of fungi. A saturated solution of copper resinate had no noticeable effect on either species. In considering these results, it should be kept in mind that a copper sulphate solution containing copper in a concentration of 0.25 p. p. m. was toxic to the conidia of both species.

The lack of pronounced toxic effect observed in the case of the copper malate solution is of special interest, since it has been pointed out by McCallan and Wilcoxon (*11*) that the conidia of *Sclerotinia fructicola* and *Glomerella cingulata*, organisms used in their experiments, apparently have the power of secreting malic and other hydroxy acids. It was their thought that when these conidia encountered bordeaux mixture residues the hydroxy acids that they secreted dissolved copper. When ionized copper comes in contact with malic acid ions, apparently a very soluble copper malate is formed, but experiments made by the present authors show that the copper is in a nonavailable form and is not particularly toxic to the conidia of the two species. The results of additional experiments dealing with combinations of copper and malic acid ions are presented in another portion of this report. The marked toxicity of normal copper maleate and basic copper maleate indicates that the copper is more available in these compounds and is readily absorbed by the conidia. Maleic acid, while extremely injurious to higher plants, appears not to be deleterious to the conidia tested. By the chromotropic test (*7*), conidia killed by these solutions were shown to have absorbed copper, indicating that the materials are either readily absorbed or that ionization of copper occurs.

The amount of copper present in the primary saturated solutions of most of these compounds is greater than in the lowest concentration of copper in the copper sulphate solutions used in the

previous experiments, yet these experiments reveal that only with certain of these materials was the toxic effect as pronounced. A possible explanation of the failure of copper silicate, a copper zeolite (2), one of the copper oxychlorides (2), and copper oxalate to show the same toxicity as a corresponding concentration of copper sulphate is that the soluble fractions contain complex ions or inactivating compounds which cause the copper to be in an unavailable form.

EXPERIMENTS WITH COPPER SULPHATE ADDED TO CULTURE MEDIA

In these experiments ionized copper, in the form of a sulphate solution, was added to water agar, Czapek's agar, and potato agar. After a considerable number of tests, it was found that the equilibrium point, i. e., the point at which enough ionized copper was added to cause a definite lethal effect, differed according to the medium used and the organism tested. The equilibrium point does not necessarily indicate that all the conidia were killed at that point. It is the point at which lethal effects are first noted and at which germination of most of the conidia is inhibited but may proceed after transfer to culture media. This is shown at the copper concentration of 185 p. p. m. (table 4) where water agar was used as the base or carrier of the copper. It will be observed that at the lower concentrations many of the conidia of both *Sclerotinia fructicola* and *Glomerella cingulata* germinated, usually with germ tubes shorter than those of the check.

TABLE 4.—*Growth responses of conidia of Sclerotinia fructicola and Glomerella cingulata when seeded for 24 hours on plain water agar to which various concentrations of copper sulphate had been added*

Concentration of copper	*Sclerotinia fructicola* [1]			*Glomerella cingulata* [2]		
	Germination	Observations	Subsequent growth [3]	Germination	Observations	Subsequent growth
P. p. m.	*Percent*		*Percent*	*Percent*		*Percent*
62	92	Long germ tubes	96	96	Medium germ tubes	100
125	66	Medium germ tubes	82	62	do	86
185	82	Short to medium tubes; all plasmolyzed.	12	76	do	87
250	12	Short germ tubes	0	56	Short germ tubes	76
312	14	do	0	68	do	78
374	10	do	0	74	do	56
434	9	do	0	72	Short to medium germ tubes.	64
500	12	do	0	58	do	62
562	10	do	0	48	do	68
625	12	do	0	52	Some cells plasmolyzed; short germ tubes.	38
Check	96	Long germ tubes	96	100	Medium to long germ tubes	100

[1] Cultures obtained from 7-day-old string bean growths.
[2] Cultures obtained from 4-day-old potato hard-agar growths.
[3] Subsequent growth determined by transferring the treated cubes of conidia-containing water agar to potato hard agar.

At concentrations nearly up to the equilibrium point, the number of conidia that germinated was much less. Especially was this true of the conidia of *Sclerotinia fructicola*, none of which were found active after being subjected for 24 hours to a concentration nearly up to the equilibrium point. A higher percentage of the conidia of *Glomerella cingulata* apparently survived contact with the copper-containing

medium at the equilibrium point, and though inhibited in their growth they continued activity when transferred to culture media. Because the percentage of subsequent survival was greatest where initial growth was most active, it appears that initial growth, by either using up or inactivating some of the copper of the solution, had lowered the copper concentration to a point where the remaining conidia were merely inhibited or not injured at all. Of course, this is the weakness of a static system. One never knows with exactness what the real concentration of any of the components of such a system may be after absorption by conidia has begun. It appears from these tests that conidia of *G. cingulata* were more active absorbers of the toxic materials than were those of *S. fructicola*.

Where *Sclerotinia fructicola* was used as the test organism, the equilibrium point for water agar was between 125 and 180 p. p. m.; for Czapek's agar, 120 to 150 p. p. m.; and for potato agar, 500 to 572 p. p. m. With *Glomerella cingulata* as the test organism, the equilibrium point for water agar was 185 to 250 p. p. m.; for Czapek's agar, 150 to 180 p. p. m.; and for potato agar, 350 to 375 p. p. m. In these static systems the initial concentration of ionized copper necessary to furnish a toxic quantity was vastly greater than that required in the dynamic system. The closeness of the equilibrium points for water agar and Czapek's agar indicates that the sugars and phosphates of the latter do not inactivate much of the copper. Potato agar was found to inactivate a high percentage of ionized copper. Water agar, though free of nutrients, apparently inactivated copper either by simple adsorption or by combination with the alcohol hydroxyl groups of the agar molecules. The complicated and changeable systems presented by the potato agar and synthetic Czapek's agar prevented their use in collecting data suitable for the determination of the equilibrium point. For this reason the water-agar systems were used to obtain the data presented in table 4. It is probable that, as was noticed when a dynamic system was used, the conidia of *Glomerella cingulata*, when transferred from nutrient media to the water-agar blocks, carried over larger quantities of inactivating substances than did the conidia of *Sclerotinia fructicola*. It appears from these experiments that it is futile to carry out tests dealing with the toxicity of metallic ions to conidia in systems where colloids and other materials have to be added to promote activity of the conidia or where activity of the conidia causes chemical changes in the substrate.

The results obtained with the copper-water-agar system appeared to indicate that for *Sclerotinia fructicola* (but not for *Glomerella cingulata*) one could fix with some degree of certainty the range of copper concentrations at which lethal and inhibitory effects on the conidia could be registered. Because experiments with the copper malate equilibrium solutions had indicated that copper ions are rendered unavailable when combined with malic acid ions, it was thought that the addition of an equal amount of malic acid to a system containing a concentration of copper ions known to be lethal to *S. fructicola* would tend to inactivate the soluble copper. Table 5 shows the results of an experiment designed to test this hypothesis. It will be noted that the addition of nearly the same amount of malic acid completely inactivated the copper at concentrations known to be toxic or inhibitory (at or near the equilibrium point). An excess of

malic acid appeared to accelerate the growth of the germ tubes, while lesser amounts allowed some of the copper to become effective. This was especially indicated when comparison was made of the effect of adding malic acid in the proportion of 50 p. p. m. to the minimum and to the maximum equilibrium concentrations respectively. Transfers to nutrient media indicated that 76 percent of the conidia survived the treatment when the solution contained 50 p. p. m. of malic acid and 125 p. p. m. of copper, whereas only 16 percent of the conidia survived when the solution contained the same amount of acid and 180 p. p. m. of copper. It appears from these studies that malic acid inactivates copper and that the copper malate molecule formed is not absorbed by the conidia of *S. fructicola*.

TABLE 5.—*Growth responses of conidia of Sclerotinia fructicola when seeded for 24 hours on plain water agar to which various concentrations of copper sulphate and malic acid had been added*

Concentration		Observations on conidia after 24 hours	Subsequent growth	Observations [1]
Copper	Malic acid			
P.p.m.	*P.p.m.*		*Percent*	
125	50	Many plasmolyzed; 26 percent with short germ tubes	76	Medium to long tubes.
125	100	A few plasmolyzed; 76 percent with medium to short germ tubes.	96	Germinated.
125	150	A few plasmolyzed; 86 percent with short germ tubes	96	Growing; tubes branched.
125	200	94 percent with medium to long germ tubes	96	Heavy growth.
125	250	96 percent with long germ tubes	------	Do.
125	300	96 percent with long to medium germ tubes	98	Do.
180	50	Mostly plasmolyzed; 15 percent with short germ tubes	16	Medium growth.
180	100	Many plasmolyzed; 18 percent show short germ tubes	76	Long germ tubes.
180	150	Only a few plasmolyzed; 66 percent show short germ tubes.	98	Do.
180	200	None plasmolyzed; 56 percent with short germ tubes	96	Do.
180	250	None plasmolyzed; 92 percent with medium to long germ tubes.	96	Branched tubes.
180	300	None plasmolyzed; 94 percent with medium and long germ tubes.	------	Do.
0	50	98 percent with long germ tubes	98	Do.
0	150	----do----	98	Heavy growth:
0	300	92 percent with medium germ tubes	------	Do.
125	0	A few plasmolyzed; 12 percent show buds or short germ tubes.	18	Medium tubes.
180	0	None germinated; mostly plasmolyzed	11	Short tubes.

[1] Observations were made 24 hours after material was transferred to potato hard agar.

DISCUSSION

These experiments have demonstrated that a very minute quantity of ionized or available copper, if kept constant by continuous replenishment, is toxic to the conidia of *Sclerotinia fructicola* and *Glomerella cingulata*, whereas without replenishment a large quantity of copper in the primary solution is necessary to bring about the same effect. Although it has not been demonstrated that the physicochemical system operating under natural conditions, when a conidium is in contact with a spray residue in a drop of rain, is exactly comparable to either of the systems that have been designated "dynamic" and "static," it seems probable that such a system is more nearly dynamic than static. Until the amount of copper coming into solution becomes less than that taken out, the toxic action of the available copper must be rapid; but it slows down as the ratio of production to absorption becomes less and less. Thus, if a conidium comes in contact with a

fresh bordeaux mixture residue the toxic action is relatively rapid; if the contact is made with a weathered and depreciated residue the action is relatively slow. In the latter case the final effect may be only inhibitory. That such changes in toxic effects actually occur has been demonstrated by Goldsworthy and Green (*7*), who showed that fresh residues of bordeaux mixture are toxic to conidia of *S. fructicola*, whereas weathered residues show varying degrees of toxicity and inhibition.

The results of experiments reported here indicate that organic compounds secreted by or adsorbed on the conidia may have the property of inactivating ionized copper in spray residues. McCallan and Wilcoxon (*11*) have demonstrated that millions of conidia may furnish traces of these materials; they have also indicated (indirectly, of course) that a single conidium must possess a very minute quantity of these materials. The results of the experiments reported here demonstrate that the materials shown by McCallan and Wilcoxon to be secreted by conidia and to have the supposed property of bringing copper into solution also have the property of changing ionized copper to inactive copper. It has been demonstrated also that the conidia of *Sclerotinia fructicola* and *Glomerella cingulata* actually survive in contact with enormous quantities of the copper compounds thus formed.

The static system used shows very definitely that organic materials such as are usually found in ordinary nutrient media are capable of inactivating considerable quantities of soluble copper. That fixation of ionic copper may also be a function of adsorption or combination with alcohol hydroxyl groups is plainly indicated by the inactivation of copper by plain water agar, where no measurable nutrient or inorganic material is present. These results indicate that a conidium in contact with a source of ionic copper may reduce the quantity of copper either by adsorption or by supplying hydroxy acids secreted by it or adsorbed on its surface.

The results obtained from the primary saturated solutions of various inorganic and organic copper compounds definitely substantiate the findings of Holland, Dunbar, and Gilligan (*10*) and Ribéreau-Gayon (*13*) in showing that the degree of solubility does not necessarily indicate the degree of toxicity. It is the available copper, i. e., copper which can be absorbed by the conidia, that is important in this respect. Thus basic copper sulphate, with a solubility of 11.5 p. p. m., was found to be more toxic than a saturated solution of copper malate, with a solubility of 1,240 p. p. m., because the available copper in basic sulphate solution is apparently greater than in copper malate solution; but it was also evident that not all the copper in the saturated solution of the basic copper sulphate exerted a lethal effect, because its toxicity was less than that of a solution of copper sulphate containing copper in a concentration of 4.0 p. p. m. This is probably true of all the saturated solutions tested, based on comparisons with the dilute, fully ionized copper sulphate solutions. Apparently the saturated solutions used in the experiments reported here are similar to those obtained by Ribéreau-Gayon (*13*) from bordeaux mixture in that only a small portion of the copper was actually ionized, or available. From the results obtained with the black copper oxide, copper zeolites, copper phosphate, copper silicate, and copper oxychlorides, it appears that none of the determined solubilities indicate the true availability of the

copper in these solutions. Although the solubilities of the two copper oxychlorides tested are greatly in excess of that shown to be toxic for the completely ionized copper sulphate, only one of them was toxic to a noticeable degree. While the red copper oxide and basic copper sulphate exert a toxic effect, it is not certain that availability and solubility are of the same order.

Although the amount of available rather than soluble copper indicates whether these compounds will be toxic when placed in contact with fungus conidia, their solubilities appear to be positively correlated with injury when in contact with the tender parts of sprayed plants. It has been found that black copper oxide, copper phosphate, copper silicate, and copper zeolites cause little, if any, injury to the leaves of sprayed plants, whereas copper oxychlorides, basic copper sulphate, red copper oxide, copper malate, copper maleate, basic copper malate, and copper oxalate are unsafe in varying degrees, depending on their solubilities. This is especially true of the malate, maleate, and basic maleate, which are the most soluble.

It therefore appears that the injurious effects of a copper-containing compound on sprayed plants may be determined by its degree of solubility and the fungicidal activities by the degree of ionization in its solution. Since most of the materials that are more or less injurious to higher plants appear to have solubilities of more than several parts per million, which is about that of alkaline bordeaux mixtures, it seems safe to predict that but few of these will be less injurious than bordeaux mixture. The ideal copper spray would apparently be one in which the concentration of dissolved copper would be not much greater than the concentration of ionized copper known to be injurious to higher plants. In such a spray the concentration of ionized copper should be high enough to be toxic to fungus spores but not high enough to cause serious injury to the sprayed plant. This concentration appears to be about 1.0 p. p. m.

SUMMARY AND CONCLUSIONS

A study is reported of the effect of low concentrations of copper ions, administered in two different ways or systems, on the activity of the conidia of *Sclerotinia fructicola* (Wint.) Rehm and *Glomerella cingulata* (Ston.) Spauld. and Schrenk.

The system referred to as "dynamic" consisted of furnishing for 24 hours a continuous supply of certain concentrations of copper ions by means of a gravity flow controlled in such a manner as to deliver the solutions to the test conidia embedded in water agar. In this system the copper concentration was kept practically constant.

The system referred to as "static" consisted of exposing the test conidia embedded in water agar to certain concentrations of copper ions added to water agar, Czapek's agar, and potato agar. In this system the copper concentration gradually decreased and was not restored.

The conidia of both species were definitely injured by solutions of copper sulphate having concentrations of ionic copper as low as 0.25 p. p. m. administered in a dynamic system.

The degree of toxicity of ionic copper is higher in a dynamic system, where the factors that favor inactivation or fixation are overcome, than in a static system, where no record of the actual concentration of ionic copper is available once the system is in operation.

The degree of toxicity of copper sulphate solution in the static system varied according to the power of the various media to fix, and consequently to inactivate, copper ions. The amount of fixation was determined by the character and composition of the media and appeared also to be greatly influenced by direct adsorption or by combination with alcohol hydroxyl groups.

Primary saturated solutions of relatively insoluble copper compounds, obtained from the first washing of 20 g of material by 2 liters of distilled water, showed differences in toxicity that apparently were correlated with the amount of available copper and not with the amount of soluble copper in the solutions.

The primary saturated solutions of cupric oxide (black), copper phosphate, copper zeolite, copper ammonium silicate, and copper silicate were nontoxic to the conidia of *Sclerotinia fructicola*, and *Glomerella cingulata;* and one of two copper oxychlorides was only slightly toxic to the conidia of *S. fructicola* and nontoxic to the conidia of *G. cingulata*.

The primary saturated solutions of cuprous oxide (red), basic copper sulphate, one of the copper oxychlorides, copper maleate, and basic copper maleate were toxic to both species.

The primary saturated solution of copper malate, having a copper solubility of 1,240 p. p. m., proved only slightly less toxic than a copper sulphate solution containing a copper concentration of 0.25 p. p. m.

The primary saturated solution of copper oxalate proved to be only slightly toxic to the conidia of *Sclerotinia fructicola* and innocuous to the conidia of *Glomerella cingulata*. Experiments with dilute concentrations of oxalic acid showed that this difference was due to the specificity of the acid.

Ionic copper in quantities sufficient to be toxic to the conidia of *Sclerotinia fructicola* could be entirely inactivated by the addition of an equivalent amount of malic acid. The acid, in low concentrations at least, was assimilated by the conidia of both species, and promoted growth.

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THE ORIGIN OF LINT AND FUZZ HAIRS OF COTTON[1]

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INTRODUCTION

The mature seeds of most varieties of upland cotton are covered with two different types of hairs, (1) the long lint hairs that are extensively used in the manufacture of fabrics, and (2) the short, thick-walled, fuzz hairs (linters) that form a dense, tangled mat close to the surface of the seed. Both lint and fuzz hairs are single-celled, tubular outgrowths that arise from the epidermal cells of the seed coat. Although the principal difference between the lint and fuzz hairs is the much greater length of the lint, it is usually difficult or impossible to draw a sharp line of demarcation between them on this basis. Very short lint hairs and long fuzz hairs may be indistinguishable. It is sometimes possible, however, to distinguish between lint and fuzz by their position on the ovules and seeds. In some varieties of cotton both the lint and fuzz hairs are restricted to specific and sometimes separate areas on the surface of the seed, giving rise to various "patterns." In the so-called naked seed varieties the fuzz hairs are entirely missing, so that removal of the lint leaves the seed coat smooth and free from hairs. In some varieties the lint and fuzz hairs are pigmented differently, and frequently pigment is present in the fuzz when it is entirely absent from the lint hairs. Another indication of an essential difference between these two kinds of hairs is the fact that the pattern of the fuzz-covered areas on the seed may be inherited independently of the lint pattern.

These facts suggest that, although lint and fuzz hairs both arise from the same layer of epidermal cells, some fundamental differences may exist between them. These differences may be the result of variations in the rate of growth of cells that originate at the same time, or lint and fuzz hairs may owe their characteristics to differences in the time or method of their origin. Although the origin and development of lint hairs has been studied, very little is known about the early history of the fuzz hairs, and the early development of the two types appears never to have been studied carefully from a comparative point of view. It is the purpose of this paper to present the results of such a comparative study of the origin and early history of lint and fuzz hairs.

PREVIOUS WORK

Cytological studies of the origin of lint hairs have been undertaken by a number of investigators. Balls (*3*)[2] states that the lint hairs all originate as single-celled outgrowths of epidermal cells which "sprout

[1] Received for publication January 29, 1938; issued May 1938. The work herein reported is a part of the joint cotton research program of the Bureau of Plant Industry and the North Carolina Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 520.

at the same stage of boll development, on or near the day when the flower opens." According to Balls (*2*), the fuzz hairs, like those of the lint, arise "in much the same way, at the same time, and from the same layer of cells." Singh (*8*) observed new fibers originating for a period of 48 hours after flowering, but Gulati (*6*) presented evidence that fibers continue to form from epidermal cells for as long as 10 days after the opening of the flower. Farr (*4*, *5*), in a cytological study based upon American upland cotton, reported that cotton hairs develop from epidermal cells for a period of 10 or 12 days after the date of flowering.

The period of fiber origin has also been studied in a very different way. Turner (*9*) reported the results of statistical studies of the number of cotton hairs upon ovules of different ages. He found a rapid increase in the number of hairs as the ovules grew older and larger. In one study, 10-day-old ovules were found to have 10,050 hairs, while 1-day-old ovules possessed only 4,530. Turner pointed out that this increase in hair number must mean that cotton hairs continue to arise after the day of flowering. Gulati (*6*) reported results indicating an even greater increase in number of hairs during the early stages of ovule growth. No distinction between lint and fuzz hairs was made in either of these studies.

In studying the variability in length of cotton fibers by statistical methods, Armstrong and Bennett (*1*) came to the conclusion that the number of fibers on the ovules increased until 25 days after the date upon which the flower opened.

Although all investigators agree that cotton hairs arise from epidermal cells on and soon after the day on which the flower opens, there is much disagreement as to the number of days after flowering during which hairs continue to be produced. In none of the studies of fiber origin has any systematic attempt been made to distinguish between lint and fuzz hairs. This study was undertaken in an effort to clarify these points and to determine as accurately as possible the early developmental history of both lint and fuzz hairs.

MATERIALS AND METHODS

Lint and fuzz hairs both arise from the same layer of epidermal cells, and they are frequently indistinguishable in appearance during the early stages of their growth. It is not always possible, therefore, to determine by observation alone whether the outward extension of any given epidermal cell is destined to become a lint hair or a fuzz hair. The fact that certain varieties of cotton have lint hairs but no fuzz and that other varieties produce lint and fuzz hairs in separate and restricted areas on the ovule surface suggested a means of identifying the two types of hairs. This study was made, therefore, by comparing the developmental histories of hairs produced upon naked (i. e., fuzz-free) seeds and pattern types (those in which lint and fuzz hairs are restricted to separate or specific areas on the seed) with seeds that produced both lint and fuzz hairs over their entire surface.

Six varieties of cotton were used in this study. Four of these—Mexican 128–6, King Naked, Cleveland Fuzzy Tip, and Nankeen Lint—are varieties of *Gossypium hirsutum* L., and two—S × P 30

(Sakel × Pima) and a Bleak Hall strain of sea island—are varieties of *G. barbadense* L.

In order to obtain a complete picture of the development of hairs in these varieties, series of ovules were collected in such a way as to give the stages of growth at 24-hour intervals. In the case of Mexican 128–6, three sets of collections were made. On July 11, 217 flowers were tagged, and ovules were collected daily thereafter for 20 days. On July 15 approximately 500 flowers were tagged, and on August 15 1,000 flowers were tagged. Daily collection from these two taggings continued for 45 days. By this method of tagging, the collection from each of the three dates of flowering constituted a reliable growth series which was representative of a particular day of flowering, and the material of successive days had experienced the same environmental conditions during the earlier periods of growth.

Because only a limited amount of material of the other varieties was available, it was necessary to tag all flowers as they opened each day and to collect the entire crop of bolls of a single variety on a day which would afford an unbroken series. In this way, Sakel × Pima material was collected on August 31, sea island on September 2, King Naked on September 15, Nankeen Lint on September 16, and Cleveland Fuzzy Tip on September 24. All collections were made in the afternoons during the summer of 1936.

After collection, the bolls were opened and the young ovules were dropped immediately into fixing agents. A number of different fixing agents were employed, but the most satisfactory results were obtained with the following formulas:

		Cc
(1)	Formalin	4
	Formic acid	4
	50-percent ethyl alcohol	92
(2)	Formalin	4
	Formic acid	4
	N-butyl alcohol saturated with water	92

After fixing, the ovules were dehydrated with n-butyl alcohol. Five- and ten-micron longitudinal sections were cut from 60° paraffin and were stained with Heidenhain's haematoxylin for maximum contrast.

RESULTS WITH VARIETIES STUDIED

KING NAKED

The King Naked variety presents the problem in its simplest form, since lint hairs alone are produced. When the lint hairs are removed from the seed, the seed coats are smooth and entirely free from fuzz (fig. 1, *A*). The mature seeds bear lint hairs over the greater portion of their surface, only the lower fifth or sixth of the seed coats being entirely devoid of fibers (fig. 1, *H*).

The simplicity of this fiber pattern is reflected at an early age by a comparable simplicity in the time and place of origin of fibers on the growing seed. On the day of flowering, the epidermal layer of the ovule gives no evidence of young fibers or fiberlike processes. Twenty-four hours later, the first fiber initials are seen developing on the chalazal half of the ovule. On the second day after flowering, these fiber initials have elongated noticeably, and no new outgrowths are ordinarily seen on this part of the ovule during this or at any subse-

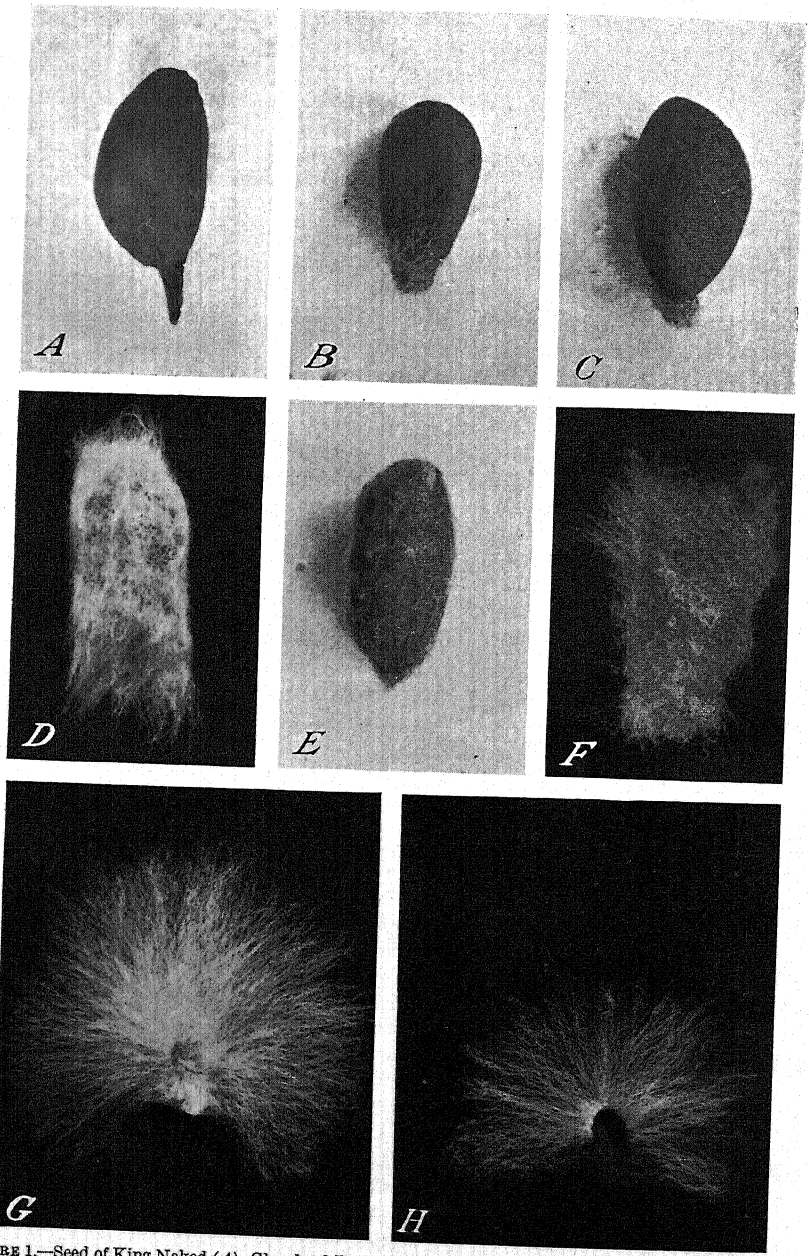


FIGURE 1.—Seed of King Naked (*A*), Cleveland Fuzzy Tip (*B*), sea island (*C*), Mexican 128–6 (*D*), Sakel × Pima (*E*), Nankeen Lint (*F*), with the lint hairs removed and the fuzz hairs combed out to show their position on the seed and relative abundance. Seed of Mexican 128–6 (*G*), King Naked (*H*), with lint hairs combed to show their distribution on the seed.

quent period. It may be seen, however, that new fibers develop in restricted numbers near the micropylar end of the ovule, but these initials serve to extend the surface range over which fibers grow rather than to contribute to the density of the fiber population of the first day. Similarly, on the third day after flowering, a few new fibers may be seen differentiating even nearer the micropylar end of the seed. Other outgrowths may continue to differentiate in this manner for several days, but the number of late fiber initials is extremely small, and in most cases the last outgrowths are seen on about the third day after flowering.

The time and place of origin of fiber initials as described here are represented diagrammatically in figure 2, *A*. In this diagram, the innermost line outlines the shape of the ovule, particularly in a submedian longitudinal section. The innermost line also represents the day of flowering and is marked 0. Each additional line to the outside represents a day after flowering up to the day of secondary wall formation, which is indicated by the outermost line. No new fibers develop after secondary wall formation has occurred. In the diagram the time and place of origin of new fibers are indicated by heavy black lines. The density of the hair population is suggested approximately by the presence of solid and broken or fragmentary lines, the solid lines representing greater numbers of hairs than the broken or fragmentary lines.

It will be noted that most of the lint hairs of the King Naked variety begin their growth on the day after flowering and that a few others differentiate during the following 2 days. Since the mature seed bears only lint hairs, it is obvious that the lint pattern is established within a period of about 3 days and that this period of lint origin falls very early in the life history of the growing ovule. Differences in fiber length may be observed from the day of fiber origin up to maturity, even in those regions of the epidermis where the fiber initials have appeared at the same time. This would seem to suggest that the length differences between mature fibers may well be due to different rates of growth.

It is interesting to note that papillate outgrowths may be observed over almost the entire surface of the ovule for 6 or 8 days after flowering, but these papillae are extremely minute and no evidence has been found that any of them subsequently elongates even as much as 5 microns.

The initiation of lint hairs in the King Naked variety is a continuous process which persists through a period of 3 or 4 days after flowering. The first hairs are formed around the chalazal end of the ovule on the day after flowering. New fibers differentiate throughout the day, and hair formation progresses from the chalazal region toward the micropylar end of the seed. By the end of the first day, half or more of the ovule may be covered by fiber initials. During the next 2 or 3 days, additional fibers are formed nearer to the micropyle and lint differentiation gradually ceases. Thus, lint hair formation progresses continuously from one end of the ovule to the other.

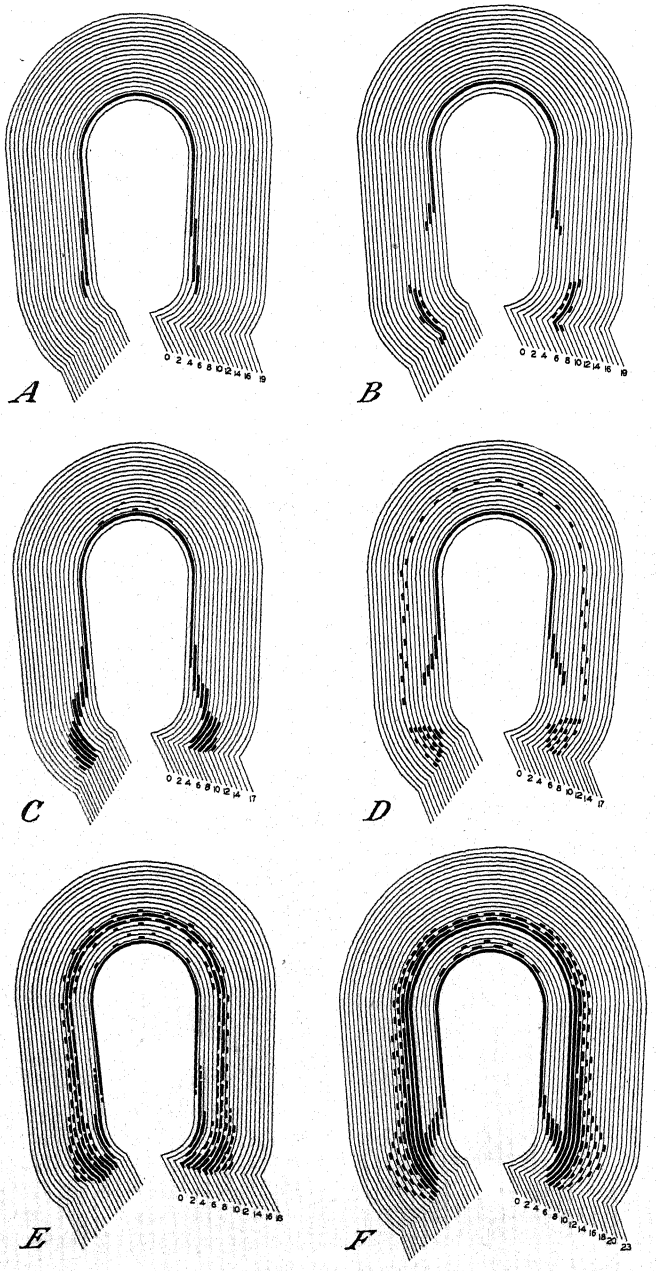


FIGURE 2.—Diagrams representing the time of origin and the distribution of the young hair initials on the surface of the ovules. The innermost line represents the surface of the ovule as seen in submedian longitudinal section on the day of flowering. Succeeding lines outside of the innermost line represent successive days after the opening of the flower. Heavy black lines indicate the position on the ovule where young hairs have been observed to originate. The relative number of hairs arising is suggested approximately by the character of the heavy black line. Where the line is solid more fibers originate than where the line is broken. The diagrams show fiber development upon ovules of varieties as follows: *A*, King Naked; *B*, sea island; *C*, Cleveland Fuzzy Tip; *D*, Sakel × Pima; *E*, Mexican 128-6; *F*, Nankeen Lint.

CLEVELAND FUZZY TIP

As the name indicates, in Cleveland Fuzzy Tip the fuzz hairs are restricted to a small area immediately surrounding the micropylar end of the seed (fig. 1, *B*). The lint hairs are usually produced over three-fourths or more of the seed surface at the chalazal end, but frequently they are limited to only about half of this portion of the seed. In this variety, therefore, both lint and fuzz hairs are present, but each hair type occupies a specific and different area on the seed coat. Although the problem is complicated in this case by the presence of both lint and fuzz, the fact that the hairs of both types are restricted to definite and separate portions of the seed coat makes it possible to determine the time and method by which each hair type originates.

On the day of flowering, the epidermis gives no evidence of fiber differentiation at any point. On the following day, outgrowths from epidermal cells of the seed coat appear in large numbers over half or more of the ovule surface at the chalazal end. As with the King Naked variety, new fibers originated for several days after flowering on portions of the ovule successively closer to the micropylar end. Unlike the King Naked variety, a few hairs were found to arise at the chalazal end of the seed on the second, third, and fourth days after flowering. The small number of these fibers is indicated by broken lines in the diagram (fig. 2, *C*).

About 6 days after flowering, proliferations from epidermal cells are formed in the region covered by fuzz in mature seeds. These outgrowths can be identified as fuzz hair initials by their position, since mature seeds bear no lint hairs in this area. As the diagram indicates, fuzz hairs continue to arise at the micropylar end of the ovule for 5 or 6 days. No hairs were observed to develop on any other portion of the ovules during this period. It seems clear, therefore, that in this variety, at least, lint and fuzz hairs originate at different times, the fuzz arising only after the complete pattern of lint hairs has been established.

The amount of lint-bearing surface in Cleveland Fuzzy Tip differs considerably from seed to seed, and this variability at maturity is reflected in a comparable variability during fiber initiation. In some instances, lint hairs differentiate only from the chalazal half of the seed, so that the final pattern is almost identical with that of sea island. In other instances, lint hairs differentiate from most of the seed surface, and since this appears to be the more common occurrence, the diagram for this variety (fig. 2, *C*) has been constructed to indicate that lint hairs arise from all of the seed surface except the fuzz-bearing portion.

MEXICAN 128–6

In the Mexican variety both lint and fuzz hairs are present over the entire surface of the mature seeds (fig. 1, *D*), so that the hair initials cannot be identified as lint or fuzz by their position on the young ovule.

On the day before flowering, a limited number of hair initials become differentiated from epidermal cells at the chalazal end of the ovule. On the day of flowering, however, hairs appear in great abundance (fig. 3, *B*) over most of the ovule surface, though few, if any, appear in the area adjacent to the micropyle (fig. 2, *E*). As in the other varieties already described, the first fibers arise at the chalazal end of the ovule, and during the same day other hairs appear

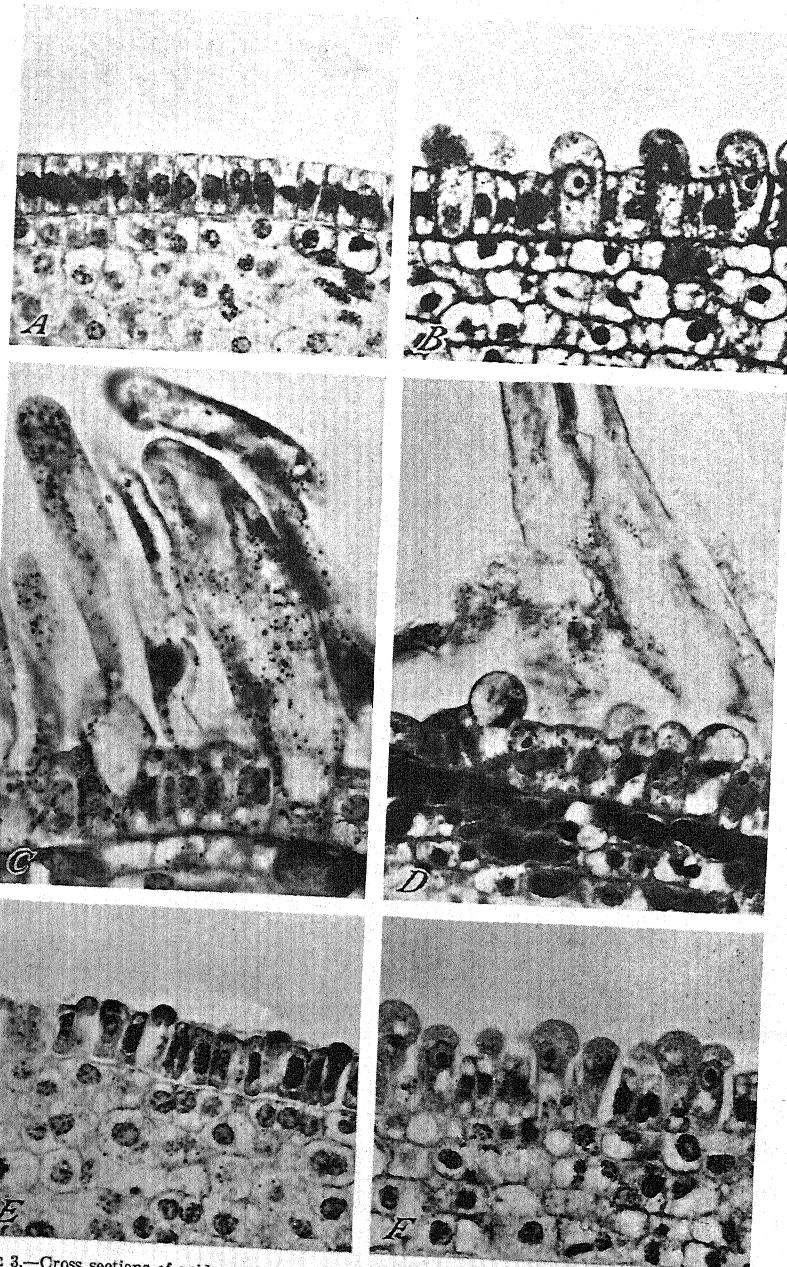


FIGURE 3.—Cross sections of epidermal layer of ovules: *A*, Mexican 128–6, the day before flowering; *B*, Mexican 128–6, the day of flowering; *C*, Mexican 128–6, 24 hours after the opening of the flower; *D*, Mexican 128–6, 5 days after flowering; *E*, sea island, 24 hours after the opening of the flower; *F*, sea island, second day after flowering.

progressively nearer the micropylar end of the ovule until it is covered with young fibers, except only the micropylar fifth or sixth of its surface area. On the day after flowering, new hairs continue to form nearer the micropylar end of the ovule and a few can be seen arising at the chalazal end as well. A small number of fibers continue to be developed from epidermal cells near the micropyle for 3 or 4 days (fig. 2, *E*). The Mexican variety differs from those previously described in having a much larger number of hair initials and also in the rapidity with which the ovule surface becomes covered with young hairs. Almost the entire surface of the young ovule bears elongating hairs within 48 hours of the day of flowering.

On the fourth or fifth day after flowering, a second outburst of fiber formation occurs (fig. 2, *E*). Fiber initials may be seen arising from newly formed epidermal cells between the bases of the first-formed hairs (fig. 3, *D*). These hair initials, like those produced on the day of flowering, first appear at the chalazal end of the ovule, and the younger hairs arise progressively nearer the micropylar end of the ovule. Within approximately 48 hours of their first appearance, almost the entire ovule surface is again covered with young hair initials. This second group of hairs continues to arise from epidermal cells of the ovule surface for a period of 4 or 5 days, and the last to be formed arise, as in previous cases, in the micropylar region. The sequence of fiber origin in the Mexican variety is shown diagrammatically in figure 2, *E*.

To interpret these observations it is necessary to recall the sequence of events described for the King Naked and the Cleveland Fuzzy Tip varieties. In these varieties it was shown that the lint hair pattern is largely established within 48 hours of the day of flowering. It was further shown that the fuzz hairs when they are present do not appear until the lint pattern had been fully determined.

A similar sequence of events apparently takes place in the Mexican variety. One set of hair initials originates within a few days of flowering, and a second set of hairs develops over the whole surface of the ovule several days later. The hairs produced on and immediately following the day of flowering correspond almost exactly in their position and time of origin to the lint hairs of the Cleveland Fuzzy Tip and King Naked varieties (fig. 2, *A* and *C*). It seems probable, therefore, that the first hair initials originating on ovules of the Mexican variety also develop into lint hairs. Following the establishment of these first-formed hairs, which are presumably the lint initials, there is an interval of several days in which very few new hairs originate.

On the fourth or fifth day after flowering, a second period of hair production is begun (fig. 2, *E*). This corresponds to the period in which the fuzz hairs develop in the Cleveland Fuzzy Tip variety. Since fuzz hairs cover the entire surface of the seed in the Mexican variety, and since the hairs arising in this second period of fiber initiation also appear over the entire surface of the ovule, it seems probable that they represent the fuzz hair initials. As will become apparent later, there is additional evidence which also indicates that the first-formed hairs develop into lint fibers and that hair initials arising several days after the opening of the flower give rise to the fuzz hairs. No significant differences in the history of hair origin and development were observed on the ovules by the three different series of Mexican studied.

NANKEEN LINT

Nankeen Lint is a variety of American upland cotton with brown lint and extremely dense fuzz. Both lint and fuzz hairs are found over the entire seed surface (fig. 1, *F*), but the fuzz is more prominent than in the other varieties studied.

As indicated in figure 2, *F*, the hairs arise in much the same manner as do those of the Mexican variety. On the day of flowering, large numbers of hairs are formed by outward extensions of the epidermal cells. Differentiation of hairs continues in regions nearer the micropylar end of the seed for 4 or 5 days after the opening of the flower. It seems highly probable that the hairs originating during the first few days after flowering give rise to lint hairs as do the King Naked and Cleveland Fuzzy Tip and the varieties of *Gossypium barbadense* that were studied. As in all fuzz-bearing varieties, the ovules of Nankeen Lint exhibit a second period of rapid hair production. This begins about 6 days after the flower opens, and active production of new hairs over the entire surface of the ovule continues for 3 or 4 days. After the tenth day relatively few new hairs are produced, and these are restricted to the micropylar end of the ovule. This prolific formation of hairs on and soon after the sixth day after flowering is exactly what would be expected, considering the varieties previously described, and suggests strongly that the fuzz hairs originate at this time. In all the varieties in which the fuzz hairs are restricted to specific areas the hairs in these regions appear about 5 or 6 days after the flower opens. This delayed appearance of hairs also occurs in Nankeen Lint, and the large number of hairs produced is clearly correlated with the dense fuzz hair population characteristic of this variety.

SEA ISLAND

Sea-island cotton, in which the lint and fuzz hairs are conspicuously restricted to specific areas on the seed, was studied as a representative of a different species of cotton (*Gossypium barbadense*). The lint is usually limited to the chalazal half or two-thirds of the seed, while the fuzz hairs form a dense growth at the extreme micropylar end (fig. 1, *C*).

On the day of flowering, some of the epidermal cells at the chalazal end of the ovule show evidence of slight protuberances. These cells are few in number and exhibit no appreciable change in size or appearance during the next 24 hours (fig. 3, *E*). On the second day after flowering, however, large numbers of hairs originate as outgrowths from epidermal cells in the chalazal half of the ovule (fig. 3, *F*). Since the papillate outgrowths observed on the day of flowering did not increase in size or number during the succeeding 24 hours, the second day after flowering is more properly regarded as the first day of fiber origin (fig. 2, *B*). On the third day after flowering, these hair initials have grown somewhat in length and new hairs may be seen arising at the micropylar margin of the zone in which hairs were formed on the second day. The area of the ovule surface on which hairs are borne is likewise increased slightly by the origin of a few new hairs on the fourth day after flowering.

Since the portion of the mature seed corresponding to the area in which these hairs are formed bears no fuzz, it is obvious that the hairs arising during the first 4 days after flowering develop into lint hairs.

On the seventh day after the opening of the flower, hairs begin to arise at the extreme micropylar end of the ovule. These hairs continue to form for 3 or 4 days. Since no lint hairs are present in this portion of the seed, these later-formed hairs must develop into fuzz hairs.

It is clear, therefore, that aside from a slower development of hair initials, sea-island cotton conforms essentially to the pattern described for the Cleveland Fuzzy Tip variety. In both cases the lint hairs appear first and the lint hair pattern is fully established upon the ovule before any of the fuzz hairs originate.

SAKEL × PIMA

The mature seeds of Sakel × Pima have both lint and fuzz hairs. The lint pattern is very similar to that of sea island and Cleveland Fuzzy Tip. The fuzz hair pattern is very different, however, for fuzz occurs irregularly over the entire surface of the seed. The fuzz hairs at the micropylar end are much like those of sea island, but on the rest of the seed the fuzz is very short and brown, adheres closely to the seed coat (fig. 1, *E*), and contains tanniferous substances.

In Sakel × Pima, hairs were observed to originate from epidermal cells on the chalazal half of the ovule on the day after the opening of the flower. New hairs continue to form during the next 3 or 4 days, but they are restricted to a small zone at the margin of the area upon which hairs were initiated during the first day after the opening of the flower (fig. 2, *D*). For reasons that will become apparent later, these first-formed hairs very probably develop into the lint hairs.

A few days after the flower opens, the epidermal cells of the seed coat become altered in appearance. This alteration appears to be due to the accumulation of mucilaginous and tanninlike compounds in the cell vacuoles. This accumulation seems to bear no relation to the age of the individual cells of the epidermal layer, for these substances are present in newly divided cells as well as in cells that are several days old. The presence of these substances in the vacuoles of the hairs is evidence that such hairs originated several days after the opening of the flower. Hairs produced 24 or 48 hours after flowering apparently do not contain these substances. Since the lint hairs of this variety are free from such mucilaginous and tanniferous deposits, it seems clear that they originated during the first few days after the flower opened.

Five or six days after flowering, hair initials appear near the micropyle, and they continue to arise for several days (fig. 2, *D*). This is the portion of the seed that bears conspicuous fuzz hairs and no lint, so it is obvious that the hair initials in this position develop into fuzz. Occasional epidermal cells on the chalazal half of the ovule begin to produce hairs on about the eighth day after flowering. These first appear near the chalazal end and arise successively nearer to the micropylar end of the seed (fig. 2, *D*). These hairs contain the mucilaginous and tanniferous deposits generally present in the epidermal layer and so can be identified with reasonable certainty as fuzz initials, since the mature fuzz hairs of this variety contain these substances, whereas they are absent from lint hairs.

In Sakel × Pima the evidence suggests clearly that the lint hairs are formed within a few days of the opening of the flower and that the

lint hair pattern is fully determined before the fuzz hairs appear. The fuzz hairs first originate about 5 days after flowering in the micropylar regions, but some 8 or 9 days after blossoming additional fuzz hairs are initiated upon the remaining surface of the ovule.

DISCUSSION

From the data that have been obtained in this study it is possible to draw certain definite conclusions and to formulate a hypothesis relative to the origin of lint and fuzz hairs. Before examining the theoretical considerations it will be advisable to set forth briefly the facts that are known.

In all the ovules examined in this study, with the single exception of the King Naked variety, on which lint hairs alone are present, young hairs were observed to originate at two different periods. The first period was on or very soon after the day of flowering, and the second period usually began 5 or 6 days after the opening of the flower. In all those varieties in which the lint hairs are present only upon restricted areas of the mature seeds, and in which it is therefore possible to identify the lint hair initials beyond question, nearly all the lint hairs were found to originate within 2 or 3 days after the opening of the flower. Few if any new lint hairs are initiated in these varieties later than 5 days after the opening of the flower. In those varieties where fuzz hairs are restricted to areas devoid of lint hairs and where the fuzz hair initials can therefore be identified with certainty, the fuzz begins to develop only after the lint pattern has been completely established. This means that the fuzz hairs originate in a period from 5 to 10 days after the flower has opened.

With the exception of Sakel × Pima, the time of lint and fuzz origin cannot be determined with certainty in the varieties that have both lint and fuzz hairs over the entire surface of the seed. The two varieties that have a dense fuzz population over the seed surface (Mexican and Nankeen Lint) exhibit many aspects of hair development that appear similar to those of the pattern types. There is, in other words, a very real analogy between their behavior in the matter of hair origin and the behavior of the varieties in which lint and fuzz can be identified by the position of the hair initials in the ovule. In such varieties or types as King Naked, Cleveland Fuzzy Tip, sea island, and Sakel × Pima, where the lint hair initials can be identified without question, they have been found to originate on or soon after the flower opens. A similar phenomenon occurs in the Mexican and Nankeen Lint varieties. By analogy, it might be assumed that these first hair initials give rise to the lint hairs. In Cleveland Fuzzy Tip, sea island, and Sakel × Pima, where the fuzz hair initials can be identified clearly, the fuzz hairs are found to originate from 6 to 10 days after flowering and well after the lint hair pattern is established. Similarly, in the Mexican and Nankeen varieties a number of hairs are observed to originate from 5 to 10 days after flowering and well after the pattern of the first-formed hairs has been determined. By analogy, these might be regarded as the fuzz hair initials. Analogies, however, are uncertain foundations upon which to erect trustworthy conclusions. It is possible, even probable, that the first set of hair initials in the Mexican and Nankeen Lint varieties is the lint hair initials, and that the second set, as in the other varieties, represents

the fuzz hair initials. In the absence of further evidence, however, it would not be possible to assert positively that the first-formed hairs produce the lint and that the second set of hair initials gives rise to the fuzz in these varieties.

Fortunately, there is evidence of a different sort which strengthens the assumption that the lint hairs develop from the hairs formed soon after the opening of the flower and that the fuzz hairs originate from the hair initials that appear 5 or more days after flowering. The statistical studies of Turner (*9*), for example, show that there is a decided increase in the total number of hairs on the cotton ovules as they grow older. One-day-old ovules of the Nandyal 14 variety were found to possess some 4,530 hairs, while 28-day ovules had 16,420 hairs. Likewise, 1-day-old ovules of the Punjab-American 4–F variety were estimated to have 5,980 hairs, in contrast to the 25,090 hairs counted upon the 28-day-old ovules. These data become highly suggestive when considered in the light of the number of lint hairs found upon mature seeds of these varieties. Nandyal 14 was found to have only 4,000 lint hairs at maturity, and Punjab-American 4–F had approximately 7,900. The number of lint hairs on mature seed, therefore, is not very different from the number of hairs found upon 1-day ovules. This is exactly what would be expected from the results of the present study, since wherever lint hairs could be positively identified at the time of their origin they were found to arise within a day or two of the day of flowering. The increase in the number of hairs upon the older ovules that is reported by Turner can be explained by pointing out that the fuzz hairs, wherever they could be positively identified at the time of their origin, have been found to originate in a period between 5 and 10 or more days after the opening of the flower. The data obtained by Turner from a purely statistical study harmonize very well with the results of the cytological study here reported.

Another line of evidence of a very different kind that supports the hypothesis of early origin of the lint and later origin of the fuzz is that reported by Harrison (*7*) as a result of his studies of metaxenia in cotton. Harrison found that hybrids between inbred Pima and Hopi strains showed definite evidence of metaxenia. One of these experiments is of interest in relation to the problem of lint and fuzz origin. Harrison reported that when Hopi pollen was used upon Pima the number of lint hairs produced upon the resulting seeds was not different from those upon inbred Pima seeds but the amount of fuzz was appreciably reduced. These results harmonize nicely with those of the present study. If lint hairs originate within 24 or 48 hours of the time of flower opening they could hardly be influenced by a hybrid embryo. Fertilization does not occur until after the lint hair pattern has been established. Fuzz hairs, however, presumably originate after fertilization has been accomplished, and the density of the fuzz pattern could, therefore, be influenced by the hybrid embryo. The results of Harrison's work may be interpreted as supporting the thesis that fuzz hairs arise after the lint pattern has been fully determined.

It seems clear from the foregoing discussion that there is considerable evidence in support of the hypothesis that lint hairs arise on or very soon after the day of flowering, while the fuzz hairs do not appear until 5 or more days after the flower opens. This is known to be true of those varieties studied in which the lint and fuzz initials could be identified by their position on the ovules. The history of hair origin

in the varieties studied in which lint and fuzz hairs could not be identified by position or appearance at the time of their origin is thoroughly consistent with early development of lint and later origin of fuzz. The early appearance of lint hairs is further suggested by statistical studies of hair density at different ages and by experiments designed to demonstrate metaxenia in cotton. The thesis that lint hairs arise early and that fuzz hairs originate only after the lint hair population has been fully determined seems, therefore, to be justified by the evidence presented.

SUMMARY

The time and place of origin of lint and fuzz hairs on the ovules of six selected varieties of cotton have been studied. The hairs in all varieties were found to have an essentially similar developmental history.

In the King Naked variety most of the lint fibers originate on the day of flowering and additional lint may differentiate during the next 2, 3, or 4 days.

In Cleveland Fuzzy Tip, lint initials differentiate in abundance on the day after flowering and others make their appearance in the following 3 or 4 days. Fuzz fibers originate continuously at the micropylar end of the ovule from about the sixth to the eleventh day.

In Mexican 128–6, hairs that are presumably the lint initials appear in abundance on the day of flowering and during the next 3 or 4 days in areas near the micropylar end of the ovule. Hairs that presumably give rise to fuzz originate on about the fourth day after flowering and continue to arise for a period of 3 or 4 days.

In Nankeen Lint, hairs believed to be the lint initials differentiate from the epidermal cells on the day of flowering and continue to form in the region of the micropyle for the next 4 or 5 days. Hairs believed to be the fuzz initials first appear in abundance on the sixth day after flowering and continue to originate for a period of about 5 or more days.

In sea island, almost all of the lint fibers originate on the second day after flowering, but a small number of others may arise on the following 2 or 3 days. The fuzz initials arise on about the seventh, eighth, and ninth days after flowering.

In Sakel × Pima, hairs that are presumably the lint initials appear on the day after flowering, and a few others develop during the next 3 or 4 days. The fuzz at the micropylar end of the seed originates between the fifth and tenth days after flowering. The fuzz on other portions of the seed apparently begins to develop on about the eighth day after the flower opens.

It is suggested that lint hairs originate when or soon after the cotton flower opens, and that the first fuzz hairs appear only after the lint population has been fully determined. It is further shown that this thesis is consistent with the results of other investigators.

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ROGAS UNICOLOR (WESM.), A BRACONID PARASITE OF THE SATIN MOTH[1] [2]

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INTRODUCTION

Rogas unicolor (Wesm.) is a European species parasitic on the satin moth (*Stilpnotia salicis* L.). It was first reared by workers of the Bureau of Entomology, United States Department of Agriculture, at Budapest, Hungary, in 1927. At that time only two adults were recovered, but in 1932 about 250 cocoons were obtained by rearing, and in 1933 and 1934 more than 7,000 cocoons were sent to the United States. Several colonies of adults have been liberated in areas infested with the satin moth in New England and Washington. Although the species is not known to have become definitely established, numerous observations have been made in the laboratory regarding its biology and morphology, and this paper gives the results of these observations.

REVIEW OF LITERATURE

Although the genus *Rogas* includes many species that are parasitic on common lepidopterous pests throughout the world, notes on their biology and habits are scattered and incomplete. *Rogas unicolor* was mentioned briefly as a parasite of *Stilpnotia salicis* in Hungary by Brown (*1*) [4] in 1931. De Fluiter (*4*) listed *Petalodes* (*Rogas*) *unicolor* (Wesm.)[5] as a parasite of *S. salicis* in Holland in 1933, giving a list of secondary parasites reared from the cocoons. *Petalodes unicolor* (Wesm.) and *Rogas unicolor* (Wesm.) are distinct, but apparently *Rogas unicolor* is the species that De Fluiter reared from the satin moth. Dustan (*2*) gave an excellent account of the internal anatomy of the full-grown larva of *Rogas hyphantriae* Gahan, which has been very helpful in this study. Fiske (*3*) gave brief notes on what he called "*Rhogas intermedias* Cresson" [5] as a parasite of *Clisiocampa* (=*Malacosoma*) *americana* F., emphasizing the role played by its hyperparasites. This species, however, is an undescribed one which was obviously misidentified. Pennington (*7*) briefly discussed the life history of *Rogas terminalis* (Cress.), parasite of *Cirphis unipuncta* Haw., and noted the production of males in parthenogenesis. Hyslop (*6*) noted *Rogas autographae* Vier. as a parasite of *Autographa gamma* L. (*californica* Speyer) and described cocoon formation. Tothill (*9*) gave a few notes on *Rogas* sp. as a parasite of *Hyphantria cunea*

[1] Received for publication Oct. 23, 1937; issued May 1938.

[2] This study was conducted under the direction of C. W. Collins at Melrose Highlands, Mass., in 1932, 1933, and 1934, and under the direction of R. C. Brown at Melrose Highlands, Mass., and New Haven, Conn., in 1935.

[3] The writer wishes to express his gratitude to W. F. Sellers for collections and notes made at Budapest, Hungary; to Karoly Mihalyi for field collections; and to Mary F. Benson for the drawing of the adult.

[4] Reference is made by number (italic) to Literature Cited p. 535.

[5] The writer is indebted to C. F. W. Muesebeck, of the Division Insect of Identification, for these notes regarding identifications.

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(Drury). Husain and Mathur (*5*) briefly noted the life history of "*Rogas testaceus* Grav. var.",[5] parasite of *Earias insulana* Boisd. and *E. fabia* Stoll. Apparently *R. testaceus* (Spin.) was intended, for Gravenhost did not describe a species of *Rogas* under this name. Hyslop (*6*), Tothill (*9, pl. 3*), and Pennington (*7*) gave good figures of the adults and cocoons of the species they worked with, and Pennington also gave a figure of the egg.

ECONOMIC IMPORTANCE

Rogas unicolor has been recorded from Belgium, Holland, Germany, and Hungary, and it seems probable that it is present in many of the places where *Stilpnotia salicis* occurs in Europe. So far as is known, it has been reared only from this host. Apparently it is of slight economic importance. De Fluiter (*4*) notes recovering it from *S. salicis* in Holland, but he does not give the number of cocoons obtained. The Bureau of Entomology conducted large-scale rearing work at several points in Austria and Hungary from 1927 through 1934 for the recovery of parasites of *S. salicis*, but *R. unicolor* was never recorded in appreciable numbers except at Budapest, Hungary. At that point from 1929 to 1934 there was a light infestation of the satin moth on a sand bar in the Danube River. This sand bar, about 1½ miles long and from 100 to 200 yards wide, supports a bushy growth of poplar ranging in height from 3 to 8 feet. In 1931, 11 cocoons were reared from 6,000 larvae. In 1932, 286 cocoons were recovered from collections totaling 26,980 larvae. In 1933, 364 cocoons were obtained from collections of 35,950 larvae, and 1,890 cocoons were collected in the field. In 1934 rearing work was discontinued, but 5,065 *R. unicolor* cocoons were collected in the field for shipment to the United States, and it was estimated that, if funds had been available, from 2,000 to 3,000 more might have been brought together.

In spite of the low percentage of parasitization by *Rogas*, the field collections showed that, barring very high secondary attack, there were enough cocoons in the field to supply a large number of adults. It was therefore felt that possibly under somewhat different conditions, such as might exist in the United States, the species could have an excellent chance of becoming more effective.

It should be pointed out at this time that cocoon collection in the field was greatly facilitated by the fact that the parasitized host larva spins a conspicuous white web, inside of which the parasite cocoon is formed.

INTRODUCTION INTO THE UNITED STATES

There were 256 *Rogas unicolor* cocoons in the first shipment sent to the United States in 1932. Out of 225 adults that emerged, 224 were females. There seemed a strong possibility, therefore, that this species normally produced females in parthenogenesis; so only females were liberated. This possibility was later confirmed, and all liberations have consisted entirely of unmated females. The number and extent of colonizations is shown in table 1.

Unfortunately for this work, during the period when liberations were made in New England the satin moth was on the decline and, although good infestations were present at the time of colonization, they practically disappeared in the following year. In Washington conditions

were far better for colonization, for a general infestation has persisted throughout the area. The adult parasite has never been recovered in the United States, although in 1934, the winter following liberations, a first-instar *Rogas* larva was dissected from a hibernating satin moth larva collected at the colonization point in Portland, Maine.

TABLE 1.—*Colonization of Rogas unicolor in the United States*

Point of liberation	Year	Females shipped	Living females liberated
		Number	*Number*
Portland, Maine	1933	500	500
Topsham, Maine	1933	500	500
South Harpswell, Maine	1934	500	500
Concord, N. H.	1933	150	150
Taunton, Mass.	1932	200	200
South Yarmouth, Mass.	1933	500	500
Woonsocket, R. I.	1934	500	500
New Haven, Conn.	1934	300	293
Olympia, Wash.	1934	432	430
Bellingham, Wash.	1934	500	497
Stanwood, Wash.	1934	500	457
Kent, Wash.	1934	500	492
Total		5,082	5,019

DESCRIPTIONS

THE ADULT

The adult (fig. 1) was described by Wesmael (*12*) in 1838 as a species of the genus *Aleiodes*. In 1863 Reinhard (*8*) placed it under Nees' genus *Rogas*.

THE EGG

The newly laid egg (fig. 2, *A*) measures 0.40 mm long and 0.11 mm wide. It is very slightly curved, bluntly rounded at the ends, and slightly broader at the cephalic extremity. The chorion is smooth and hyaline except for a slightly roughened area at the cephalic end. A conspicuous round, light area may be observed near the caudal end of the embryo. This is the gonad of the first-instar larva.

THE LARVA

There are five larval instars. The first and the last are distinct, but the intermediate instars differ little from one another except in size and mandibular measurements. Each of the first four instars has an extremely delicate skin, and often the next larval instar may be distinguished as it develops under the skin about to be molted.

FIRST INSTAR

The first-instar larva (fig. 2, *B*) averages 0.40 mm long and 0.12 mm wide when hatched. It attains a size of about 0.60 mm long and 0.15 mm wide preparatory to hibernation, and in the spring develops to almost three times this size before molting to the second instar. It possesses a head and 13 well-differentiated segments. The cuticle is smooth, without spines or setae, and there is no tracheal system. The head (fig. 2, *C*) has a well-developed hypostoma and pleurostoma.[6] The epistoma, instead of constituting a continuous arch

[6] The terminology of the head structures is that of Vance and Smith (*10*).

above the mouth opening, is represented by two well-developed struts which run vertically above the superior pleurostomal rami. The maxillae and labium are defined by lightly sclerotized markings, bearing four pairs of setae. There are four sensoria just above the mouth opening. The mandibles (fig. 2, *D*) are simple, sharply curved hooks, measuring 0.02 mm. The internal anatomy is similar to that of the full-grown larva, which will be described fully, except that in the first instar the gonads are well developed and conspicuous. They are circular in outline and occupy a large part of segments 10 and 11 above the hind-intestine.

Second Instar

Second-instar larvae range from 1.8 mm long and 0.56 mm wide to 3.75 mm long and 0.95 mm wide. The tracheal system is developed, and,

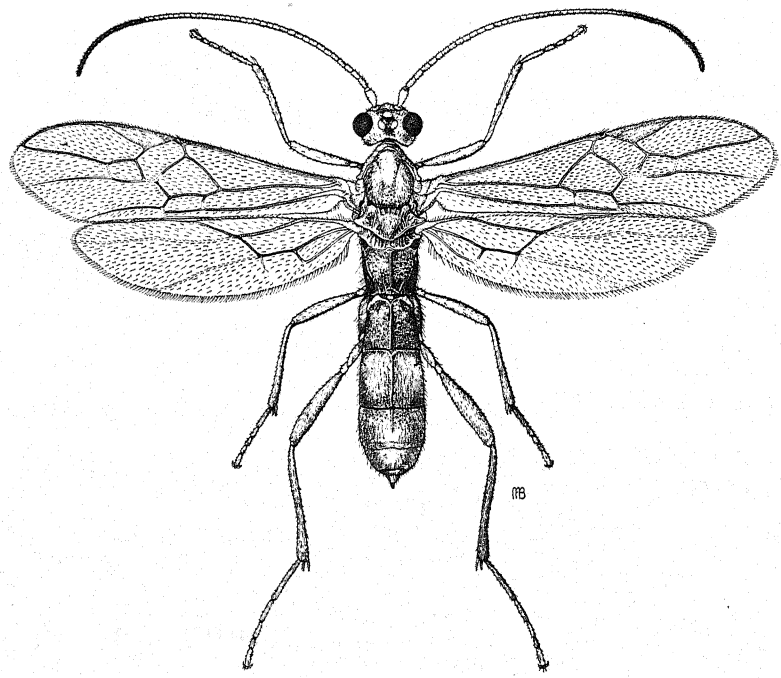


FIGURE 1.—*Rogas unicolor*. Adult female, × 9.

although there are no spiracles, there are spiracular stubs in the second and third thoracic and first eight abdominal segments. The head structures are weakly sclerotized. The hypostoma is well defined, but the mandibular and maxillary lobes are weakly shown and the epistoma forms a barely discernible arch over the mouth. The mandibles have the same general form as those of the full-grown larva. They measure 0.04 mm. The gonads are inconspicuous.

Third Instar

Third-instar larvae exhibit but slight differences from those of the preceding instar. They range from 4.0 mm long and 1.0 mm wide to 5.0 mm long and 1.32 mm wide. The mandibles measure 0.06 mm.

FOURTH INSTAR

Fourth-instar larvae range from 5.0 mm long and 1.5 mm wide to 9.0 mm long and 2.0 mm wide. There are still no open spiracles, the integument is bare, and except for the hypostoma and pleurostoma

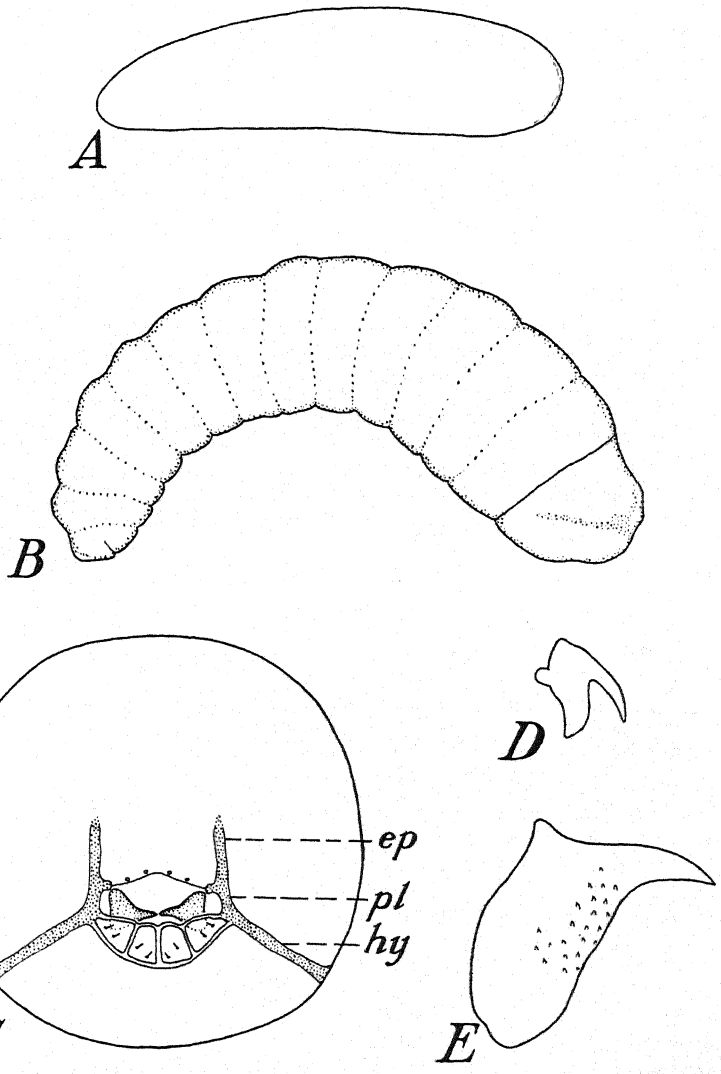


FIGURE 2.—*Rogas unicolor*: *A*, Egg, × 152. *B*, First-instar larva, lateral view, × 150. *C*, Head of first-instar larva, front view; *ep*, epistoma; *pl*, pleurostoma; *hy*, hypostoma, × 458. *D*, First-instar mandible, × 650. *E*, Last-instar mandible, × 235.

the head skeleton is practically undefined. The mandibles measure 0.10 mm.

FIFTH INSTAR

The full-grown larva (fig. 3, *A*) measures about 10 mm long and 2.5 mm wide. It is yellowish white, and when quiet assumes the char-

acteristic shape, with head curved downward, as shown in the figure. The first eight abdominal segments bear prominent lateral folds. The integument bears tiny cuticular spines as indicated. The last abdominal segment bears, in addition, several (about 12) pairs of sensory setae. Sensory setae are also present on the other segments,

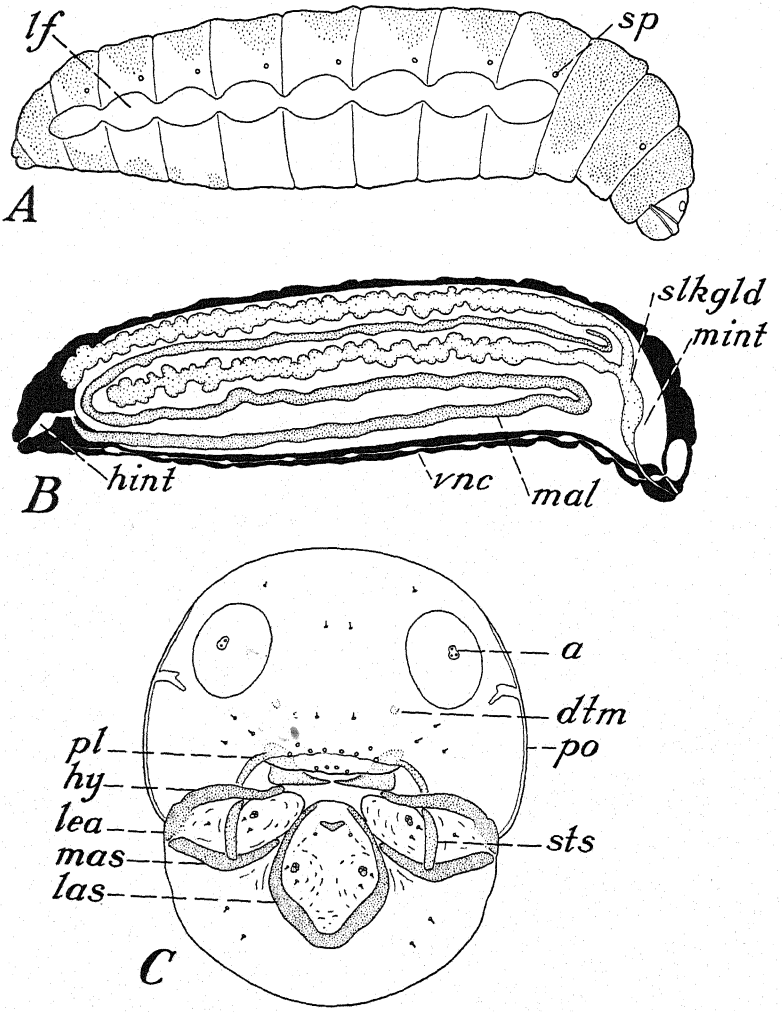


FIGURE 3.—*Rogas unicolor: A*, Full-grown larva; *sp*, spiracle; *lf*, lateral fold, × 11. *B*, Full-grown larva, internal anatomy; *slkgld*, silk gland; *mint*, midintestine; *mal*, malpighian tube; *vnc*, ventral nerve cord; *hint*, hindintestine, × 11. *C*, Head of full-grown larva, front view; *a*, antenna; *dtm*, dorsal tentorial mark; *po*, postocciput; *sts*, stipital sclerome; *pl*, pleurostoma; *hy*, hypostoma; *lea*, lateral epicranial angle; *mas*, maxillary sclerome; *las*, labial sclerome, × 72.

but owing to similarity with the cuticular spines and unevenness of distribution their exact number and location were not determined.

The head, seen from the front (fig. 3, *C*), presents well-defined characters. The antennal rudiments are somewhat darkened areas containing two or three minute papillae. They are surrounded by

large oval areas, the antennal foramina. The postocciput is well defined laterally. Just below the antennal foramina pronounced grooves extend inward from the postocciput. These grooves may be the temporal fossae. The hypostoma widens and divides at the lateral epicranial angles, where one side is fused to the strong transverse tentorial bar. The stipital sclerome is apparently not attached to the hypostoma. It sets off the heavy fold of the maxillary region. The maxillary and labial palpi each have two slightly raised round sensoria. The mandibles (fig. 2, *E*) are simple and broadly oval at the base. On the inner side of the basal part they bear a number of minute spines. They measure 0.14 mm. An area on the ventral portion of the head is armed with cuticular spines.

The tracheal system is composed of a pair of lateral trunks, which give rise to a main dorsal and a main ventral branch in each segment except the head and the last segment. There are nine pairs of spiracles. A pair occurs on the mesothoracic segment and a pair on each of the first eight abdominal segments. They are joined to the longitudinal trunks by short spiracular branches. A spiracular stub occurs in the metathoracic segment, but there is no spiracle. The mesothoracic spiracle measures 0.04 mm in diameter. The main dorsal branches in the first segment are connected, and in each of the first eight abdominal segments small tracheae arising from the main ventral branches unite to form a ventral transverse commissure. The head and the last segment receive branches from the main longitudinal trunks. The main dorsal and ventral branches give off numerous small tracheae, which branch profusely. Some small tracheae also arise from the longitudinal trunks.

The digestive system (fig. 3, *B*) is similar to that described by Dustan (*2*) for *Rogas hyphantriae* Gahan, although larvae of *R. unicolor* were not sectioned, and many of the details described for *R. hyphantriae* were not studied.

The alimentary canal consists of a short, slender foreintestine, a large midintestine, occupying most of the body cavity from the first to the eighth abdominal segment, and a short hindintestine. The contents of the midintestine are brownish pink. The hindintestine is composed of three well-defined areas—a short bulbous section just behind the midintestine, an even shorter constricted section, and a large saclike rectum. The malpighian tubes are attached to the first section. The rectum is about three times as wide as it is high. The salivary glands are well-developed tubes lying on each side of the body. They unite in a short, common duct, which has a U-shaped opening on the floor of the mouth. The common duct divides into two tubes at the posterior border of the head. Each tube extends upward to the third segment, where it bifurcates. Each branch possesses many actively secreting cells that are grouped in conspicuous oval nodules joined along the main tube. Posteriorly these nodules are more numerous, and consequently the tubes become considerably widened. There is only one pair of malpighian tubes, but they are exceedingly long and are attached very closely to one another at the base of the hindintestine. They do not have a common lumen as in *Rogas hyphantriae*. They separate almost immediately, running just above the ventral nerve cord to the third thoracic segment, where they turn, run posteriorly almost as far as the hind intestine, turn again, and run

anteriorly almost to the second thoracic segment, where there is another short turn before they end. They are for the most part straight-sided tubes, but here and there a slight swelling occurs.

The brain and the nerve cord are distinct. The ventral nerve chain includes 11 double ganglia, the terminal one being in the eighth abdominal segment. The abdominal ganglia are fused. Each is located at the anterior border of the segment, and gives off a pair of long nerve fibers running posteriorly and laterally through the segment. The terminal ganglion is evidently a fusion of the last three segments, for it sends out three pairs of nerves.

The heart, or dorsal blood vessel, may be distinguished readily in living specimens. It runs from the first segment just behind the brain abruptly dorsad to the median line, and along this line to the twelfth segment.

The urate cells are conspicuous just beneath the cuticle. They are irregular in shape, semiopaque, and occur from the second to the seventh abdominal segments, inclusive.

The histoblasts of the various external organs are easily distinguishable, since they lie just underneath the cuticle and are semiopaque. The antennal histoblasts are situated behind the antennal rudiments; those of the legs are on the venter of the thoracic segments; those of the wings are found laterally on the second and third thoracic segments; and the histoblasts of the female genitalia are located ventrally in the eighth and ninth abdominal segments.

The rudiments of the ovaries are located in the seventh abdominal segment. They consist of two spindle-shaped bodies with long, narrow stalks ventrally and numerous tiny filaments dorsally. The stalks are attached to the ventral wall on each side of the ventral nerve chain, and the filaments are joined dorsally.

THE COCOON AND PUPA

Characteristic of the genus, the cocoon of *Rogas unicolor* is formed inside the dead host larva's skin. The contents of the host larva are almost entirely consumed, and the parasite spins a light, but tough, brown cocoon within the host skin. As the skin dries it forms a dry, taut case, conforming closely to the shape of the full-grown parasite larva. The head of the dead host larva remains attached to the skin and is curved downward.

The pupa is of the usual hymenopterous type. It is oriented toward the caudal end of the host larva.

BIOLOGY

Rogas unicolor has one generation a year. It passes the winter as a first-instar larva within the hibernating host larva. Development proceeds slowly in the spring, and the parasite larva does not become full grown until the host larva is in the penultimate instar. Under insectary conditions in New England on an average 12 days is spent in a cocoon. *Rogas* adults issue from June 21 to July 7, the majority appearing the end of June. Probably issuance would be somewhat later under field conditions. Satin moth eggs are deposited late in June and in July. They hatch in about 15 days, and 5 to 6 days are spent in the first instar. *Rogas* females attack the second-instar larvae. Females issuing the first of July probably do not find an

abundant supply of suitable host material for 2 or 3 weeks, but undoubtedly some larvae are present soon after they appear.

As in other species of *Rogas*, the adults issue by gnawing an irregular hole through the dorsal side of the cocoon near the caudal extremity of the dead host's larval skin. In one or two instances the emergence hole has been found on the venter. The adults are rather inactive, slow-moving insects. At the laboratory they were confined in glass-topped wooden boxes and fed dry lump sugar and a honey solution (1 part honey to 5 parts water) held on absorbent cotton or sponges. When placed in a cool, dark room they lived on an average 34 days. When placed in 8-inch glass vials for continuous reproductive work, they lived a somewhat shorter time. Six females used in reproduction-capacity work in 1933 lived on an average 22 days, with a minimum of 12 and a maximum of 32 days. In 1934 the females used in similar work were held for 3 to 4 weeks before suitable host larvae could be provided, and they lived on an average only 8 days after beginning oviposition.

As already indicated, experiments have shown that *Rogas unicolor* normally produces females by parthenogenesis. A number of larvae were attacked at the laboratory by both mated and unmated females. In all 90 adults were reared, and 89 of them were females. The one male was the progeny of an unfertilized female. Apparently about the same proportion of females are produced under natural conditions, for during a 3-year period out of 5,492 adult *Rogas* issuing from cocoons received from Europe only 40 were males.

Although females are normally produced in parthenogenesis, the sexes mate fairly readily. Eight pairs averaged 30 seconds in coitu.

Rogas females oviposit very readily in second-instar host larvae. First-instar larvae are so small that the ovipositor cannot be successfully inserted. When a *Rogas* female comes near a host larva, she quickly touches it with her antennae and moves toward it with abdomen curved downward ready to strike. If the larva stops, she often stands still over it a moment and then prods it gently with her abdomen. When the larva is moving, she inserts her ovipositor by a quick forward thrust of the abdomen. Oviposition requires about a second, and the parasite then hurries away. Since the parasite's ovipositor is very short and the host larva small, the larva is often lifted up, and frequently rolls itself into a tight ball. If the larva rolls up before the ovipositor is actually inserted, the parasite may make use of her long antennae and palpi to hold it in position for attack. The parasite shows no discrimination, frequently attacking the same host larva a number of times, but she always walks away a short distance after each egg is laid, and is therefore not immediately attracted to the same larva. Observations conducted during two seasons on isolated females indicated that *Rogas unicolor* has a rather high reproductive capacity. The maximum number of eggs laid by one female was 322, but when this female was dissected there were still a number of eggs in her ovaries and ovarioles. Each ovary has two very long ovarioles, and it is very difficult to count all the eggs present. This particular female had at least 100 more eggs in the process of formation, and it is quite possible that more might have been developed. The maximum number of eggs laid by a single female over a 2-day period was 88.

When it is first deposited, the egg apparently floats freely in the body cavity of the host larva, for it floats out very readily upon dissection. After about 24 hours, however, it seems to be lightly attached to various host organs, but most frequently to the silk glands, malpighian tubes, or fat body. The egg hatches in about 53 hours. The tiny parasite larva floats freely in the body cavity of the host. It ingests a small quantity of the host body fluids, increasing slightly in size before hibernation. In the spring it develops very slowly, first-instar larvae having been dissected out as late as June. The next three instars are completed within 3 or 4 days, and it is difficult to find a larva of an intermediate instar without traces of the next one already apparent through the thin skin. Several days are spent in the last larval instar. The contents of the host larva are almost entirely consumed and then the parasite cocoon is spun. During the greater part of this instar the head of the parasite is oriented similarly to that of the host. As the cocoon is spun the parasite reverses its position completely, and pupation takes place with the head of the parasite toward the posterior segments of the host and its venter toward the host dorsum. Upon completion of the cocoon the larva voids its meconium and pupation takes place within about 24 hours.

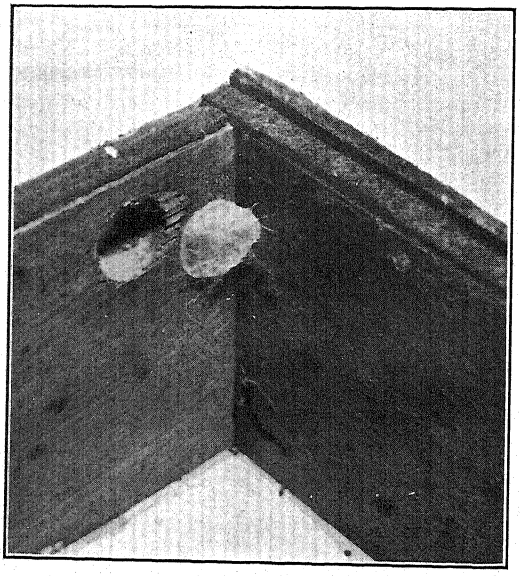


FIGURE 4.—Web spun by *Stilpnotia salicis* larva parasitized by *Rogas unicolor*.

Vickery (*11*) found that just before cocoon formation the larva of *Rogas laphygmae* Vier. cuts through the skin and pushes the fluids and material it does not eat out of the host larva's body. The drying of these fluids attaches the cocoon to the plant on which it is resting. Fiske (*3*) reported that larvae of "*Rhogas intermedias* Cresson" attach their cocoons with a bit of brown silk extruded from a puncture on the ventral thoracic surface. *Rogas unicolor* does not cut through the host larval skin. The cocoons are dry and unattached.

EFFECT OF PARASITE ON HOST

The growth of *Stilpnotia salicis* larvae parasitized by *Rogas unicolor* is considerably retarded. There is no apparent difference in parasitized and unparasitized larvae until the penultimate instar. Those that are unparasitized develop rapidly through the last instar and begin to pupate, while those that are parasitized fail to molt to the last instar and become very sluggish. Finally, about the time the parasite enters the fourth instar, the caterpillar seeks a sheltered

place and spins a dense, white web that is usually oval (fig. 4). In the field this web is usually spun between leaves. The parasite cocoon is formed inside this protective covering.

HYPERPARASITES

Unfortunately *Rogas unicolor* is attacked by a number of hyperparasites. Fourteen species have been reared from cocoons collected from the field at Budapest, and De Fluiter (*4*) records four species reared from "*Petalodes* (*Rhogas*) *unicolor* (Wesm.)" in Holland. *Dibrachys cavus* (Walk.) was the most important hyperparasite at Budapest. During 1933 and 1934 it parasitized more individuals than all the other species together. It is gregarious, and during the 2-year period on an average 15 adults were reared from each *Rogas* cocoon. This species is present in New England, and Fiske (*3*) has recorded *Dibrachys boucheanus* (=*cavus* Walk.) as an important parasite of "*Rhogas intermedias* (Cresson)."

Data concerning the hyperparasites reared from *Rogas unicolor* cocoons collected at Budapest have been summarized in table 2.

In 1933 hyperparasites issued from 14 percent of the cocoons that produced adults, and in 1934 from 17 percent. Since this material was collected in the field, it gives some indication of natural conditions, but probably a much higher percentage would have been destroyed if the cocoons had been left there until the adults emerged.

TABLE 2.—*Parasitization of field-collected cocoons of Rogas unicolor from Budapest, Hungary, in 1933 and 1934*

Species of parasite [1]	1933			1934		
	Parasitized cocoons	Parasites issued	Average parasites per cocoon	Parasitized cocoons	Parasites issued	Average parasites per cocoon
Itoplectis scanica Vill	25	25	1	98	98	1
Theronia atalantae Poda	19	19	1	65	65	1
Hemiteles areator Panz	2	2	1	6	6	1
Mesochorus tuberculiger Thoms	34	34	1	65	65	1
Mesochorus pallidus Brischke	1	1	1			
Brachymeria intermedia (Nees)	2	2	1	12	12	1
Catolaccus ater Ratz	9	9	1	4	4	1
Monodontomerus dentipes Boh	4	12	3	6	20	3+
Tetrastichus rapo Walk	1	1	1			
Pleurotropis facialis Gir.[2]	1	16	16	15	341	22+
Cirrospilus pictus (Nees)				2	30	15
Dibrachys cavus (Walk.)[3]	125	1,875	15	405	6,049	15
Eurytoma sp	1	1	1			
Eupteromalus sp.[4] (gregarious)	12	210	17+	18	242	13
Eupteromalus sp.[4] (solitary)	4	4	1	29	29	1
Total killed by parasites	240			725		
Adults issued	1,528			3,583		
Dead from unknown causes	307			757		
Total cocoons collected	2,075			5,065		

[1] Determinations made by C. F. W. Muesebeck, A. B. Gahan, and R. A. Cushman, of the Bureau of Entomology and Plant Quarantine, Division of Insect Identification.

[2] 1 cocoon produced 16 *Pleurotropis* and 4 *Dibrachys*.

[3] 1 cocoon produced 3 *Dibrachys* and 1 *Eupteromalus*.

[4] A. B. Gahan, of the Bureau of Entomology and Plant Quarantine, Division of Insect Identification, states that this species of *Eupteromalus* is "very close" to *E. nidulans* Foerst., and that the large solitary specimens are probably large specimens of the same species.

De Fluiter (4) reared *Dibrachys? boucheanus* Ratz. (=*cavus* Walk.?), *Eurytoma appendigaster* Boh., *Habrocytus emerus* (Ratz.), and *Mesochorus marginatus* Thoms. from *Rogas* cocoons in Holland. *D. cavus* is the only one of these species that was also present at Budapest.

Rogas spends about 12 days in the cocoon, but all the hyperparasites except the two species of *Mesochorus* attack this stage. *Mesochorus tuberculiger* Thoms. attacks the tiny hibernating *Rogas* inside the *Stilpnotia salicis* larva. *Mesochorus* females pay practically no attention to unparasitized *S. salicis*, but upon encountering a parasitized larva the hyperparasite strokes it rapidly with its antennae and jabs it repeatedly with its ovipositor. Upon dissection the attacked *Rogas* larva is found to contain a tiny gourd-shaped *Mesochorus* egg.

FACTORS LIMITING THE EFFECTIVENESS OF THIS PARASITE

The only obvious factor limiting the effectiveness of *Rogas unicolor* as a parasite of *Stilpnotia salicis* is the hyperparasitism. There are numerous parasites, however, that are apparently just as severely attacked by hyperparasites, which prove to be far more effective as control factors of their respective hosts. Although *S. salicis* has been reared at many points in Europe, *R. unicolor* has been recorded in appreciable numbers only at Budapest, Hungary, and at Wageningen, Holland. It seems that there must be some unknown factors, possibly climatic, that keep it from becoming a more important parasite of this host in Europe.

SUMMARY

Rogas unicolor (Wesm.) is a braconid parasite of minor importance on *Stilpnotia salicis* L. in Europe. In 1933 and 1934 about 7,000 cocoons were collected at Budapest, Hungary, and sent to the United States. Adults were colonized in New England and in Washington, but the species has not yet been definitely recovered.

The various stages of the parasite are described in detail.

Rogas unicolor has one generation a year. The winter is passed as a first-instar larva within the hibernating host larva. Development proceeds slowly in the spring, and the parasite becomes full grown when the host larva is in the penultimate instar. The cocoon is formed inside the skin of the dead host larva. About 12 days is spent in the cocoon, and adults issue the last of June. Parthenogenetic reproduction results in female progeny, although an occasional male appears. A maximum of 322 eggs was obtained from a single female.

Host larvae that are parasitized by *Rogas unicolor* are considerably retarded in growth. Just before the parasite becomes full grown, the host larva spins a dense white web, within which the parasite cocoon is formed.

Rogas unicolor is attacked by a number of hyperparasites. Fourteen species were reared from *Rogas* cocoons collected in the field at Budapest, Hungary. The most important hyperparasite was *Dibrachys cavus* (Walk.), which has been noted by Fiske as an important parasite of "*Rhogas intermedias* (Cresson)" in New England. Hyperparasitism is the most apparent factor limiting the effectiveness of this parasite, although climate or other, unknown factors may actually be more important.

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TRANSMISSION OF SUGARCANE MOSAIC BY THE GREEN BUG (TOXOPTERA GRAMINUM ROND.)[1]

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INTRODUCTION

In 1920 Brandes[3] reported experiments showing that sugarcane mosaic was transferred by the corn leaf aphid (*Aphis maidis* Fitch). This remained the only proved vector until 1936, when the present authors[4] reported a series of successful transfers with the rusty plum aphid (*Hysteroneura setariae* (Thos.)) and stated that the green bug (*Toxoptera graminum* Rond.) had been observed to transfer mosaic in two cases. Additional experiments, conducted during 1936, gave further proof that the green bug is a vector, and the results of these experiments are reported herein.

DISTRIBUTION AND HABITS OF THE GREEN BUG

Toxoptera graminum is a species of world-wide distribution, having been reported from India, Africa, Australia, and Europe, as well as from North America. It has been found in nearly all parts of the United States. In the Mississippi River Basin it causes heavy damage to small-grain crops, principally wheat and oats. This aphid has not been reported as feeding on sugarcane elsewhere than in Louisiana and not until recently in that State.

In the sugarcane section of Louisiana this aphid has been observed chiefly on sugarcane, Johnson grass (*Sorghum halepense*), crabgrass (*Digitaria sanguinalis*), *Panicum dichotomiflorum*, and *P. reptans*. In many fields it has been found in numbers about equal to those of *Aphis maidis* on sugarcane, but generally it is not so abundant. The green bug occurs in greatest abundance on crabgrass. Large numbers have also been seen on Johnson grass, which is sometimes found growing in sugarcane fields but more frequently on ditchbanks and headlands surrounding the fields. None of these aphids were ever found on oats growing alongside a sugarcane field in which there was a heavy infestation on crabgrass.

Both winged and wingless forms are commonly found in the whorls of sugarcane plants, where they have undoubtedly been mistaken for *Aphis maidis* in the past. *Toxoptera graminum* also feeds on the other parts of the plant. It is sometimes observed feeding at the tips of the leaves on the under side. Since the green color of this aphid is more nearly like that of sugarcane than is the color of any of the other aphids occurring on sugarcane, it is the most difficult species to find.

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[2] The authors gratefully acknowledge helpful suggestions from P. N. Annand, of the Bureau of Entomology and Plant Quarantine, and R. D. Rands, of the Bureau of Plant Industry.
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ATTEMPTED TRANSFERS FROM MOSAIC SUGARCANE TO HEALTHY SUGARCANE

Parallel experiments were conducted in which *Toxoptera graminum* was compared with *Aphis maidis*, which still appears to be the most efficient vector of sugarcane mosaic. Since Co. 281 is one of the generally planted varieties suffering serious injury from mosaic in Louisiana, it was used in these experiments.

Single bud cuttings of mosaic cane, to be used as a source of inoculum, and pedigreed healthy cane were planted in 4-inch pots of sterilized soil, sprouted in incubators, and held in an insect-proof greenhouse until ready for use. When they were from 3 to 6 inches high, the mosaic plants were transferred to cloth and glass insect-proof cages in the insectary. These cages were either 15 by 28 by 49 or 16 by 30 by 49 inches high. Aphids were collected from nonmosaic host plants and placed on the mosaic plants at the rate of about 50 per plant. Separate cages were maintained for each species of aphid. Thirty viruliferous aphids were transferred to each healthy cane plant 24 to 26 hours later, and these plants were then placed in insectary cages of the type just described. In from 5 to 7 days these plants were fumigated. Check plants were given the same treatment except for the absence of aphids from the cages.

TABLE 1.—*Summary of mosaic-transmission experiments with Toxoptera graminum and Aphis maidis in 1936*

Date of transfer of insects	Species of aphid used	Healthy plants used	Healthy plants developing mosaic	Period of exposure before mosaic symptoms appeared
		Number	*Number*	*Days*
Apr. 8	*T. graminum*	32	4	15 (2), 19 (2).[1]
	A. maidis	23	4	14, 17 (2), 26.
	Check	7	0	
Apr. 15	*T. graminum*	2	0	
Apr. 27	*T. graminum*	10	1	18.
	A. maidis	6	2	12 and 14.
	Check	6	0	
Apr. 29	*T. graminum*	23	2	10 and 12.
	A. maidis	21	8	12, 14 (2), 16, 19, 21, 26, 54.
	Check	6	0	
May 1	*T. graminum*	28	4	14 (3) and 27.
	A. maidis	32	20	13 (3), 14 (7), 15 (4), 18, 20 (3), 22, 25.
	Check	10	0	
May 4	*T. graminum*	15	4	11, 16 (2), 21.
	A. maidis	17	3	16, 46 (2).
	Check	7	0	
May 6	*T. graminum*	28	2	19 and 45.
	A. maidis	11	1	45.
	Check	5	0	
May 8	*T. graminum*	28	3	17 and 33 (2).
	Check	15	0	
May 29	*T. graminum*	1	0	
	A. maidis	8	1	18.
	Check	7	0	
June 2	*T. graminum*	5	1	14.
	A. maidis	6	1	14.
	Check	7	0	

[1] Numbers in parentheses indicate the number of plants that developed mosaic after the number of days that are given immediately preceding the parentheses.

Immediately after fumigation all plants with the surrounding soil were transferred to flats in the greenhouse, where they remained located on ant-proof benches until discarded. No aphids were found in the greenhouse, although frequent examinations were made. Plants

were examined at daily or, in some cases, 2-day intervals, for the first appearance of mosaic symptoms. The plants were given a liberal application of nitrogen fertilizer and kept under as favorable growing conditions as possible. All plants used in a given experiment were derived from the same source, were of the same age, and were grown under similar conditions.

The results of these experiments are shown in table 1. It may be noted that there were 21 mosaic transfers to originally healthy cane in the 172 plants exposed to viruliferous *Toxoptera graminum* as compared with 40 out of 124 healthy plants similarly infested with *Aphis maidis* in parallel experiments. No mosaic appeared in the 70 check plants or in any plants of similar origin growing in the greenhouse in other experiments.

ATTEMPTED TRANSFERS FROM MOSAIC CRABGRASS TO HEALTHY SUGARCANE

Crabgrass is the most abundant of the grasses that show symptoms of mosaic in sugarcane fields. Since *Toxoptera graminum* has often been found in large numbers on this grass, a few experiments were conducted to determine whether this aphid is capable of transferring mosaic from crabgrass to sugarcane. Experiments were conducted in cages as in previous tests.

In an experiment begun on July 29, 30 individuals of *Toxoptera graminum* from mosaic crabgrass were transferred to each of 36 healthy plants of P. O. J. 234 sugarcane. Seven days later the infested plants were fumigated. No live aphids were observed on the plants prior to fumigation; they had died soon after being transferred as a result of parasitization and high temperatures. There was no transfer of mosaic.

On August 10, 30 *Toxoptera graminum* aphids from crabgrass were transferred to each of 14 healthy plants of P. O. J. 234 sugarcane. Although the aphids died soon after the transfer, one plant showed mosaic 18 days after exposure to aphids. None of the 14 parallel check plants, given similar treatment except for exposure to aphids, developed mosaic.

On July 31, 30 individuals of *Toxoptera graminum* were transferred from mosaic crabgrass to each of 4 healthy crabgrass plants. The plants were fumigated 5 days later. None of these plants developed mosaic.

DISCUSSION AND CONCLUSIONS

In experiments conducted in 1936 *Toxoptera graminum* transferred mosaic from diseased to healthy sugarcane plants in 21 cases. These transfers, together with 2 transfers obtained in 1935 and previously reported and 1 from mosaic crabgrass, make a total of 24 transfers of mosaic to healthy sugarcane by this aphid. This establishes *T. graminum* as a vector of sugarcane mosaic.

From results reported here it is calculated that 12 percent of the healthy plants developed mosaic when infested with viruliferous *Toxoptera graminum*, and 32 percent developed mosaic when infested with *Aphis maidis* in parallel experiments. On the basis of these limited observations and of insect-population counts in and around sugarcane fields, it appears that *T. graminum* is of less importance than *A. maidis* as a vector of sugarcane mosaic.

Proof of mosaic transfer by the green bug is important, however, since it gives a third known insect vector of this disease in Louisiana. The time required for the appearance of mosaic symptoms, as seen from data given in the table, was about the same with *Aphis maidis* and *Toxoptera graminum*. Although the data on this point are limited and therefore not conclusive, such similarity would be expected, since both species ordinarily feed in the same place, that is, in the central whorl of the plant.

The discovery of this additional vector further complicates the problem of decreasing mosaic spread by means of vector control. Even though the green bug may not usually be a major agent in the spread of mosaic, it will have to be considered in any control program, because its increase in numbers under certain weather, host-plant, or other conditions may cause it to become an important vector of mosaic disease in sugarcane.

SUMMARY

Data reported in 1920 showed that the corn leaf aphid (*Aphis maidis* Fitch) was a vector of sugarcane mosaic. In 1936 the rusty plum aphid (*Hysteroneura setariae* (Thos.)) was shown to be a vector of the same disease, and two transfers of mosaic by the green bug (*Toxoptera graminum* Rond.) were reported.

The green bug is fairly generally distributed throughout the world. It is found in nearly all parts of the United States, and causes heavy injury to small grains in the Mississippi River Valley. These aphids feed upon various parts of sugarcane plants that are above ground and on a number of other grasses commonly found in and around sugarcane fields.

In a series of experiments conducted during 1936, 30 *Toxoptera graminum* from infected sugarcane plants were transferred to each of 172 healthy plants, and 21 of these developed mosaic. In a parallel experiment 40 of 124 healthy plants exposed to viruliferous *Aphis maidis* developed mosaic symptoms. One transfer of the mosaic resulted from the green bug from infected crabgrass being colonized on healthy sugarcane.

The data indicate that the green bug is not, in general, so important in field transfer of sugarcane mosaic as either of the other two vectors, but that it may be the most important transfer agent in certain fields.

A MOSAIC DISEASE OF CHINESE CABBAGE [1]

By C. M. TOMPKINS, *assistant plant pathologist*, and H. REX THOMAS, formerly *graduate assistant in plant pathology, California Agricultural Experiment Station* [2]

INTRODUCTION

In October 1934, a mosaic disease of Chinese cabbage (*Brassica pe-tsai* Bailey) was observed in the northern part of the San Joaquin Valley and later in the Salinas Valley of California. Although natural infection in this and subsequent years varied from 60 to 100 percent, negligible losses resulted and the heads usually attained marketable size. Preliminary mechanical inoculations to Chinese cabbage and certain other cruciferous test plants in the greenhouse were successful and indicated that this virus was apparently different from the cauliflower mosaic, annual stock mosaic, and cabbage black ring viruses previously reported from this State (*11*, *12*, *13*).[3] In addition to presenting the results of studies of the disease, this paper briefly compares the Chinese cabbage mosaic virus with certain other crucifer viruses.

REVIEW OF LITERATURE

Apparently the first published record of a mosaic disease on Chinese cabbage was contributed by Schultz (*9*) in 1921. He noted that infected plants were dwarfed, with mottled, ruffled, and distorted leaves. Mustard (*B. japonica* Sieb.) and turnip (*B. rapa* L.) in nearby areas were similarly affected. By means of juice inoculations, he obtained intertransmission between these hosts after 20 to 30 days; symptoms developed in 12 to 30 days when infective green peach aphids were transferred to healthy plants. Simultaneously, Gardner and Kendrick (*5*) described a mosaic disease on turnip, and it seems highly probable that their virus was identical with that studied by Schultz (*9*).

In 1924 Kunkel (*7*) found intracellular bodies in mosaic-diseased leaves of Chinese cabbage plants which had earlier been collected in Honolulu gardens. In a later communication,[4] he stated that the disease "caused some distortion of leaves and stunting of the plants but was not particularly severe."

Under greenhouse temperatures ranging from 70° to 80° F., Clayton (*1*) determined that Chinese cabbage could be readily infected by juice inoculations with the rutabaga mosaic virus. The plants showed inconspicuous mottling, stunting, and streak symptoms.

A mosaic disease of Chinese cabbage, turnip, and mustard was observed in Fukuoka, Japan, in 1927 by Takimoto (*10*). Crop losses frequently approached 30 percent. Characteristic symptoms consisted of dwarfed, mottled, and deformed leaves. The virus was shown to be transmitted by means of aphids (species not given).

[1] Received for publication November 29, 1937; issued May 1938.
[2] The writers are indebted to Profs. Ralph E. Smith and M. W. Gardner for advice and assistance and to Prof. B. A. Madson and W. W. Mackie for providing greenhouse space and facilities. Valuable assistance in the greenhouse work was rendered by employees of the Federal Works Progress Administration.
[3] Reference is made by number (italic) to Literature Cited, p. 551.
[4] Letter dated December 20, 1934, from L. O. Kunkel, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, N. J.

Cauliflower (*Brassica oleracea* L. var. *botrytis* L.) was infected by means of juice inoculations, but not cabbage (*B. oleracea* L. var. *capitata* L.) or radish (*Raphanus sativus* L.). Since Gardner and Kendrick (*5*) were also unable to obtain infection of radish with the turnip mosaic virus, it is possible that the Japanese virus may be closely related to strains of the turnip virus found in the United States.

Further references to the occurrence of a mosaic disease of Chinese cabbage in Japan were published in 1932 and 1933 by Fukushi (*4*) and Hino (*6*), respectively.

Accordingly to Weber (*14*), a mosaic disease of Chinese cabbage is of minor importance in Florida, causing negligible losses. The identity of the virus was not mentioned.

In Chinese gardens in Manila, P. I., 30 to 50 percent infection of Chinese cabbage plants was not uncommon, according to Fajardo (*3*).

SYMPTOMS OF THE DISEASE

As observed under greenhouse conditions, the first symptoms of the mosaic disease of Chinese cabbage consist of a pronounced systemic clearing of the veins, which generally commences at or near the base of the leaf and gradually spreads over the entire leaf (fig. 1, *A*, *B*). After 3 to 4 weeks the early symptoms on the young, inner leaves commence to change gradually into a very conspicuous, coarse type of mottling (fig. 1, *C*), in marked contrast to healthy leaves (fig. 1, *D*). The irregular, light- and dark-green areas between the veins, which give rise to the mottled appearance, cause little or no distortion of the leaf surfaces. Raised, dark-green islands on the leaves, which characterize the mosaic diseases of certain plants, were not observed on any of several thousand artificially-infected plants used during the coarse of these studies. Usually the older, outer leaves of infected plants continue to show clearing of the veins without other change, and this condition persists until they turn yellow and die. The disease causes a mild stunting of the entire plant, which may not be apparent, however, if infection occurs late.

MATERIALS AND METHODS

The Chinese cabbage mosaic virus which was used in the experimental studies originated from a mature, systemically infected Chinese cabbage plant grown in the Salinas Valley. After the virus had been established on young Chinese cabbage plants in the greenhouse by mechanical inoculation, successive transfers were made to healthy seedlings at frequent intervals in order to provide a constant source of fresh inoculum.

Temperatures in the greenhouse in which these tests were conducted ranged from 13° to 19° C. The methods followed were essentially those described in a recent paper (*12*). Mechanical inoculations were made by dusting the leaves with powdered carborundum (600 mesh) and rubbing lightly with a piece of cotton soaked with the juice from a diseased plant.

Chinese pe-tsai cabbage seedlings, four to six leaves, served as test plants for both property studies and recovery of the virus. The experimental host range of the virus was determined by means of mechanical inoculations in the greenhouse.

TRANSMISSION

Preliminary inoculations of Chinese cabbage by rubbing the leaves with expressed juice gave approximately 50-percent infection. Further tests resulted in 100 percent infection when carborundum (*8*) supplemented this method. The use of carborundum tended slightly to decrease the incubation period, which was determined as ranging from 13 to 22 days.

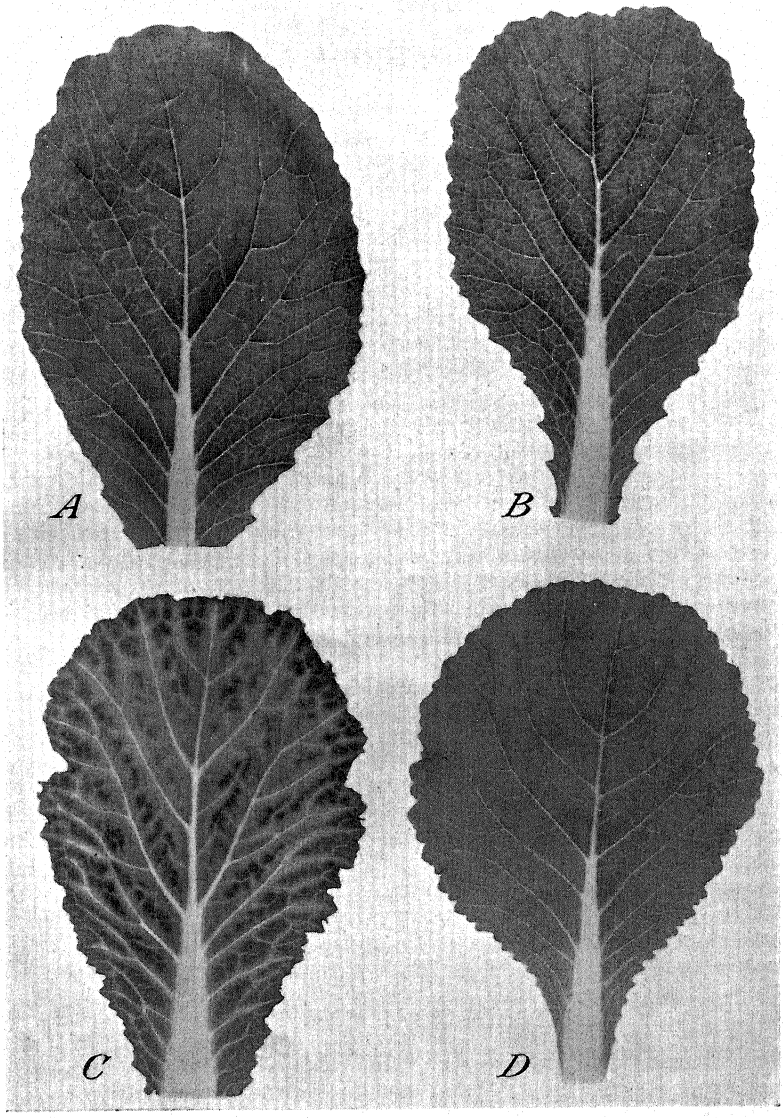


FIGURE 1.—Symptoms produced by the Chinese cabbage mosaic virus on Chinese cabbage leaves by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Early stage of vein clearing; *B*, advanced stage of vein clearing; *C*, late stage of infection showing coarse type of mottling; *D*, noninoculated control.

Studies on insect transmission of this disease involved the use of the cabbage aphid (*Brevicoryne brassicae* (L.)) and the green peach aphid (*Myzus persicae* (Sulzer)), both of which, according to Essig (*2*), breed naturally on cabbage, cauliflower, and other cultivated and wild cruciferous plants. Schultz (*9*) transmitted the crucifer virus with which he worked to Chinese cabbage by means of the green peach aphid only.

Noninfective cabbage and green peach aphids [5] were colonized on healthy cauliflower and sugar-beet (*Beta vulgaris* L.) plants under cloth cages in the greenhouse. When the population had increased sufficiently, detached leaves with aphids were placed on diseased Chinese cabbage plants under cages to permit of natural migration. After the aphids had fed for 24 to 48 hours, transfers in lots of approximately 20 infective aphids each were made to individual, healthy Chinese cabbage seedlings, as outlined above. Chinese cabbage seedlings infested with noninfective aphids served as controls, plus a number of noninoculated plants which were free from aphids.

In all, 60 Chinese cabbage seedlings were tested with infective cabbage aphids, of which 56 showed typical symptoms of the disease within 14 to 23 days. Of 20 plants tested with infective green peach aphids, 17 became diseased after 17 to 23 days. The virus was recovered from all infected plants by the carborundum method. All controls remained healthy.

Tests for seed transmission were limited to seed obtained from 19 diseased Chinese cabbage plants which had previously been inoculated by mechanical means in the greenhouse. Seeds were planted in a flat of autoclaved soil. After 21 days, 477 plants were examined, all of which were healthy, indicating that the virus is probably not carried in the seed. Schultz (*9*) planted mustard seed derived from mosaic mustard plants and concluded that there was no evidence of seed transmission.

SUSCEPTIBILITY OF CHINESE CABBAGE VARIETIES TO ARTIFICIAL INFECTION

Young seedlings of the following varieties of Chinese cabbage were tested by mechanical inoculation in the greenhouse to determine their susceptibility to infection: Chinese pe-tsai (several strains), Chinese Chicken, Chinese Chihli, and three Japanese selections of pe-tsai (Hootooren, Nagasaki Hakusai, and Tsuzita Hakusai). A suitable number of plants of each variety was reserved for controls. All varieties proved highly susceptible and the incubation period for each was within the limits of 13 to 22 days.

EXPERIMENTAL HOST RANGE

Inoculation tests indicate that the virus becomes systemic only in cruciferous plants, though local lesions were produced on two species of the Solanaceae (table 1). A brief description of the symptoms produced on each host is given in table 1, and characteristic symptoms on different hosts are shown in figures 2, *A*, *B*, *C*, *D*, and figure 5, *A*.

[5] Aphids identified by E. O. Essig, as noninfective cabbage and green peach aphids, were kindly supplied by H. H. Severin and J. H. Freitag, Division of Entomology and Parasitology.

TABLE 1.—*Plants susceptible to the Chinese cabbage mosaic virus, as indicated by mechanical inoculation of greenhouse-grown seedlings, and symptoms characteristic of infection*

Family	Species and common name	Symptoms produced
Cruciferae	*Brassica oleracea* L. var. *botrytis* L. (cauliflower—variety February).	Systemic. Numerous chlorotic rings, best seen by transmitted light.
	B. oleracea L. var. *capitata* L. (cabbage—var. Winter Colma).	Systemic. Very coarse, yellowish vein banding.
	B. napus L. (rape)	Systemic. Coarse mottling.
	B. rapa L. (turnip—var. Purple Top White Globe).	Systemic. Fine type of vein clearing and mottling.
	Matthiola bicornis DC. (evening scented stock).	Systemic. Coarse mottling, with marked chlorosis and rosetting of the terminal growth.
	M. incana R. Br. var. *annua* Voss (annual stock—var. Fiery Blood Red).	Systemic. Fine type of mottling and flower breaking.
	Lunaria annua L. (honesty)	Systemic. Vein clearing.
	Malcomia maritima R. Br. (Virginian stock).	Do.
	Raphanus sativus L. (radish—var. White Icicle).	Systemic. Vein clearing and mottling.
	Capsella bursa-pastoris (L.) Medic. (shepherds-purse).	Systemic. Mottling.
	B. adpressa Boiss.	Systemic. Chlorotic ring pattern, producing somewhat of a mottled effect.
	B. arvensis (L.) Ktze. (charlock)	Systemic. Vein clearing.
Solanaceae	*Nicotiana glutinosa* L.	Not systemic. Symptoms on inoculated leaves only, consisting of numerous chlorotic rings.
	N. tabacum L. (tobacco—var. Turkish)	Not systemic. Symptoms on inoculated leaves only, consisting of numerous chlorotic rings with dark-green edges.

Unsuccessful attempts were made to transmit the virus to 54 species of plants representing 46 genera in 27 families, as follows: Corn (*Zea mays* L.) var. Golden Bantam; oat (*Avena sativa* L.); rhubarb (*Rheum rhaponticum* L.); lambsquarters or white pigweed (*Chenopodium album* L.); sowbane or nettle leaf goosefoot (*Chenopodium murale* L.); spinach (*Spinacia oleracea* L.) var. Bloomsdale; sweet-william (*Dianthus barbatus* L.); babysbreath (*Gypsophila paniculata* L.); rocket larkspur (*Delphinium ajacis* L.); hyprid delphinium (*D. cultorum* Voss) Blackmore & Langdon strain; Iceland poppy (*Papaver nudicaule* L.); oriental poppy (*P. orientale* L.); *Brassica integrifolia* O. E. Schulz var. *chevalieri* R. Porteres; leaf or Chinese mustard (*B. juncea* Coss); white mustard (*B. alba* (L.) Boiss.); mignonette (*Reseda odorata* L.); *Geum chiloense* Balb.; garden pea (*Pisum sativum* L.) var. Alderman; broadbean (*Vicia faba* L.); storksbill (*Pelargonium zonale* Willd.); garden nasturtium (*Tropaeolum majus* L.); castor-bean (*Ricinus communis* L.); pansy (*Viola tricolor* L.); fibrous-rooted begonia (*Begonia semperflorens* Link and Otto); *Clarkia elegans* Dougl.; *Godetia grandiflora* Lindl.; celery (*Apium graveolens* L.) var. Golden Self Blanching; forget-me-not (*Myosotis alpestris* Schmidt); common heliotrope (*Heliotropium peruvianum* L.); garden verbena (*Verbena hybrida* Voss); flowering sage (*Salvia farinacea* Benth.); *Solanum aviculare* Forst.; potato (*S. tuberosum* L.) var. Katahdin; tomato (*Lycospersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner; currant tomato (*L. pimpinellifolium* Dunal); *Nicotiana langsdorffii* Weinm.; jimsonweed (*Datura stramonium* L.); petunia (*Petunia hybrida* Hort.); snapdragon (*Antirrhinum majus* L.); beardtongue (*Pentstemon barbatus* Nutt.); mourning bride (*Scabiosa atropurpurea* L.); cucumber (*Cucumis sativus* L.); canterbury-bells (*Campanula medium* L.); lobelia (*Lobelia hybrida* Hort.); dandelion (*Taraxacum officinale* Weber); head lettuce (*Lactuca sativa* L. var.

capitata Hort.) var. New York and Tom Thumb; annual marguerite (*Chrysanthemum coronarium* L.); China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt-resistant; English daisy

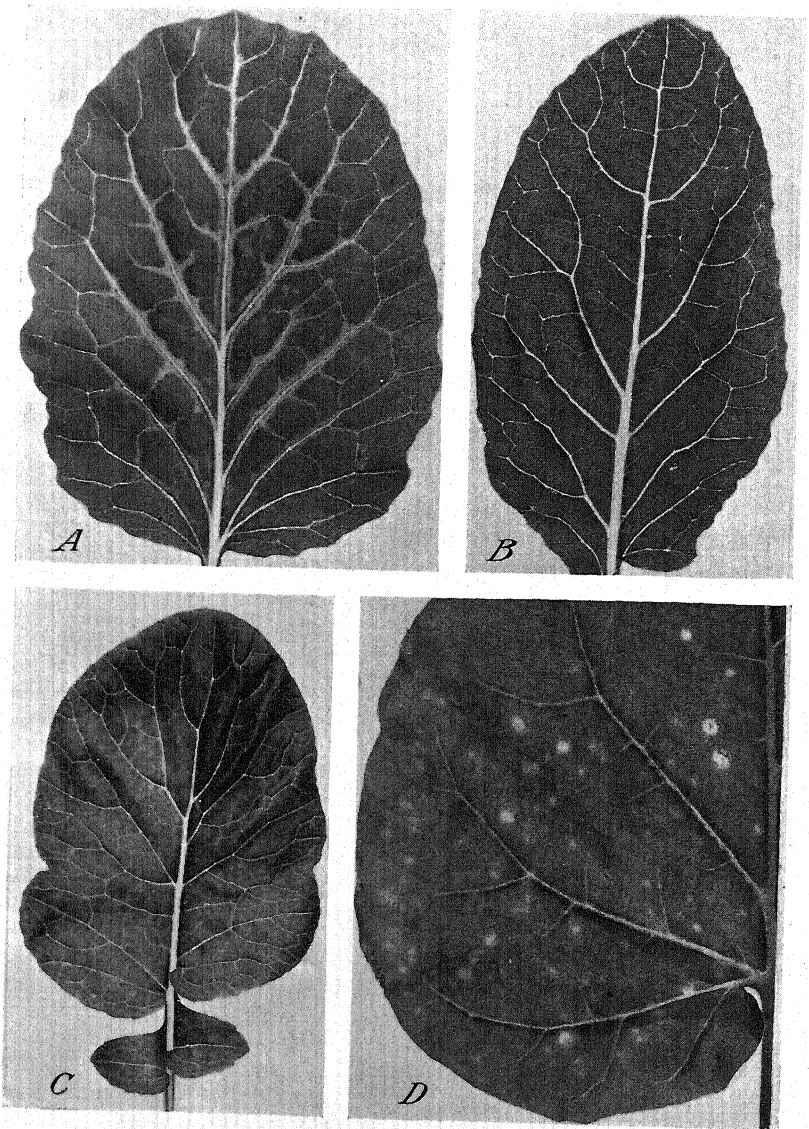


FIGURE 2.—Symptoms produced by the Chinese cabbage mosaic virus on leaves of certain plants by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Winter Colma cabbage; *B*, February cauliflower; *C*, White Icicle radish; *D*, *Nicotiana glutinosa*.

(*Bellis perennis* L.); African marigold (*Tagetes erecta* L.); French marigold (*T. patula* L.); *Gaillardia pulchella* Foug. var. *picta* Gray; winter Cape-marigold (*Dimorphotheca aurantiaca* DC.); and cineraria (*Senecio cruentus* DC.).

PROPERTIES OF THE VIRUS

In table 2, the results of property studies of the Chinese cabbage mosaic virus are given. All virus samples, consisting of 2 cc of undiluted juice from diseased plants, were contained in small, stoppered, thin-walled test tubes. For longevity tests, virus samples were stored at a constant temperature of 22° C. The virus retained its infectivity for 72 hours but was inactivated after 96 hours. The inactivation temperature lies between 73° and 75° for 10-minute exposure, which closely approaches that established for the cauliflower mosaic virus (*12*). Infection was obtained when the virus was diluted to 1 to 5,000, but higher dilutions inactivated it. In each of the three tests, 25 noninoculated plants served as controls, and they continued healthy throughout.

TABLE 2.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the Chinese cabbage mosaic virus*

[25 plants inoculated in 5 trials in each case]

LONGEVITY IN VITRO, 22° C.							
Aged (hours)	Trials	Plants inoculated	Plants infected	Aged (hours)	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
0	5	25	21	72	5	25	3
4	5	25	16	96	5	25	0
8	5	25	8	120	5	25	0
24	5	25	14	144	5	25	0
48	5	25	8	168	5	25	0
INACTIVATION TEMPERATURE (10 MINUTES)							
Temperature (°C.)	Trials	Plants inoculated	Plants infected	Temperature (°C.)	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
50	5	25	25	73	5	25	8
55	5	25	25	75	5	25	0
60	5	25	25	80	5	25	0
65	5	25	16	(1)	5	25	25
70	5	25	14				
TOLERANCE TO DILUTION							
Dilution	Trials	Plants inoculated	Plants infected	Dilution	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
0	5	25	24	1:2000	5	25	2
1:10	5	25	22	1:3000	5	25	2
1:25	5	25	15	1:4000	5	25	2
1:100	5	25	12	1:5000	5	25	1
1:200	5	25	6	1:6000	5	25	0
1:500	5	25	4	1:7000	5	25	0
1:1000	5	25	2	1:8000	5	25	0

[1] Not treated.

DESCRIPTION OF THE CHINESE CABBAGE MOSAIC VIRUS

Transmitted in greenhouse tests by means of *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer). Transmissible by mechanical inoculation with expressed juice, with or without powdered carborundum. Incubation period 13 to 22 days. Resistance to aging in vitro between 3 and 4 days. Inactivation temperature between 73° and 75° C. for 10-minute exposure. Tolerance to dilution approximately 1 to 5,000. Chinese cabbage (*Brassica pe-tsai* Bailey) and certain other vegetable and ornamental crucifers susceptible. On Chinese cabbage, symptoms consist of slight stunting of the plant; vein clearing followed by mottling, with but slight distortion of leaves and no necrotic lesions or midrib curvature. Local lesions produced on *Nicotiana tabacum* L. and *N. glutinosa* L. but not on *N. langsdorffii* Weinm.

COMPARISON OF THE CHINESE CABBAGE MOSAIC VIRUS WITH CERTAIN OTHER CRUCIFER VIRUSES

In the course of these studies, the Chinese cabbage mosaic virus was compared with the cauliflower mosaic and turnip mosaic (from New York) viruses (*12*) for symptom expression by means of parallel

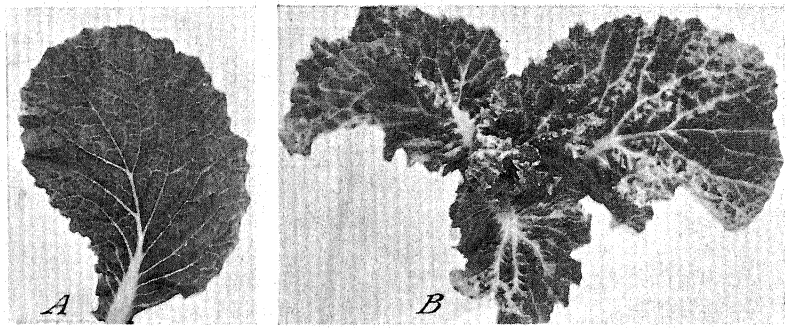


FIGURE 3.—Symptoms produced by the cauliflower mosaic virus on leaves of Chinese cabbage by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Leaf distortion and curvature of the midrib are characteristic effects; *B*, stunting of the plant.

mechanical inoculations made simultaneously to Chinese cabbage, Winter Colma cabbage, February cauliflower, and Purple Top White Globe turnip seedlings in the greenhouse.

On Chinese cabbage, the symptoms produced by the Chinese cabbage mosaic virus have been described. The cauliflower mosaic virus causes systemic infection after 20 days, the symptoms consisting of a conspicuous clearing of the veins which in the early stages cannot be distinguished from the vein-clearing symptom characteristic of the early effects of the Chinese cabbage mosaic virus. Within a few days, however, vein clearing is supplemented by curvature of the midrib, with considerable distortion and ruffling of the leaf (fig. 3, *A*). Vein clearing persists as the leaves become older, but no instances of mottling were observed. The entire plant is severely stunted (fig. 3, *B*). These symptoms are specific to the cauliflower mosaic virus and as such offer a ready means of identification.

Systemic infection of Chinese cabbage plants with the turnip mosaic virus occurred 21 days after inoculation. Symptoms were most pronounced on the outer or older leaves of the plant and consisted of a coarse, yellowish type of vein banding (fig. 4). Since Schultz (*9*) observed mosaic mottling on infected Chinese cabbage plants in both the field and greenhouse, rather than vein clearing as observed by

the writers, the existence of strains of the turnip mosaic virus is indicated.

On Winter Colma cabbage, the symptoms produced by the Chinese cabbage mosaic virus consisted of a very coarse, yellowish, vein banding (fig. 2, *A*). The cauliflower mosaic virus caused vein clearing only (*12*, *fig. 3*, *C*), while the turnip mosaic virus from New York induced light-green to yellow lesions whose edges finally became necrotic (*12*, *fig. 5*, *A*). Cabbage proved to be an excellent differential host.

On February cauliflower, the Chinese cabbage mosaic virus causes systemic infection, consisting of scattered chlorotic rings with dark-green centers. These rings, with an average diameter of one-eighth of an inch, do not become necrotic with age and, being barely discernible, are best viewed by means of transmitted light (fig. 2, *B*). The symptoms produced by the cauliflower mosaic virus on this host consist of vein clearing, followed by vein banding, mottling, and necrotic lesions (*12*, *fig. 1*, *A*, *B*, *C*). Although the turnip mosaic virus also produced a chlorotic green to yellow ring pattern on cauliflower (*12*, *fig. 5*, *B*), the lesions cannot be confused with those caused by the Chinese cabbage mosaic virus. The latter do not turn yellow and are much more diffuse (fig. 2, *B*).

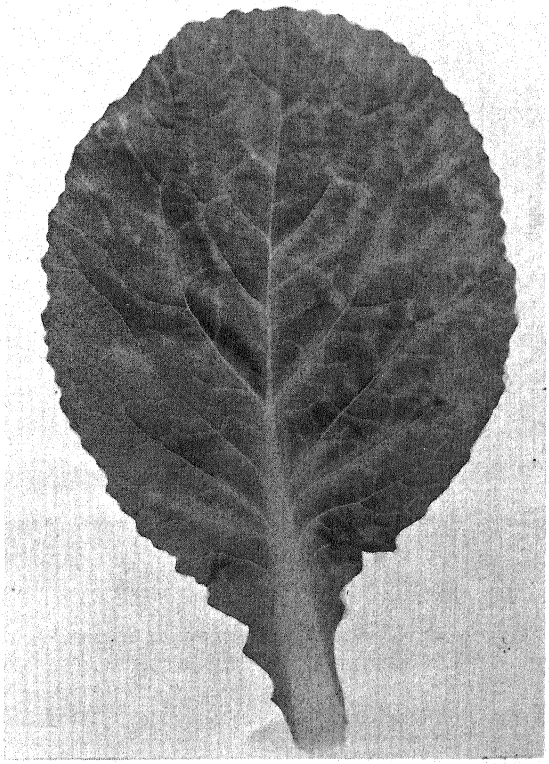


FIGURE 4.—Symptoms produced by a turnip mosaic virus from New York on leaves of Chinese cabbage by mechanical inoculation in the greenhouse at 13° to 19° C., showing coarse, yellow vein banding.

Purple Top White Globe turnip seedlings proved to be the best differential host for ready separation of the three viruses on the basis of symptoms. The Chinese cabbage mosaic virus caused systemic vein clearing after 20 days, followed by a very fine type of mottling (fig. 5, *A*). The cauliflower mosaic virus caused vein clearing but no mottling (*12*). A very coarse, contrastive type of mottling, accompanied by leaf ruffling and raised areas or dark-green blisters (fig. 5, *B*) characterized infection by the turnip mosaic virus.

A comparison of the three viruses as to properties cannot be made since no data are yet available for the turnip mosaic virus from New York. If the Chinese cabbage mosaic virus is compared with the

cauliflower mosaic virus (*12*), it will be observed that the longevity in vitro of the former is 3 days as against 15 days for the latter; the inactivation temperatures tend to approach each other. A dilution tolerance of 1 to 5,000 was obtained for the Chinese cabbage mosaic virus as compared to 1 to 2,000 for the cauliflower mosaic virus.

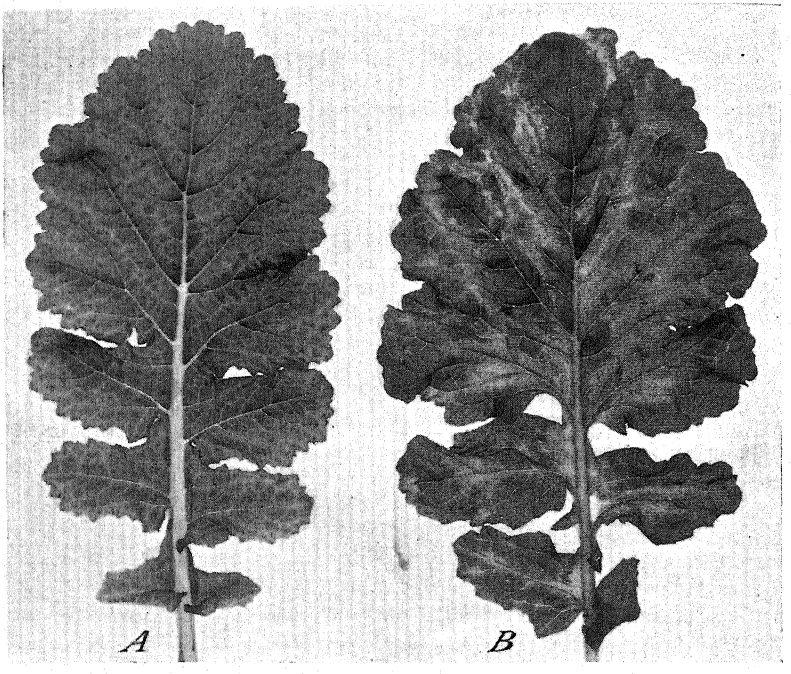


FIGURE 5.—Symptoms produced on leaves of Purple Top White Globe turnip by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, By the Chinese cabbage mosaic virus, which induces vein clearing and mottling; *B*, by a turnip virus from New York which causes a coarse, bold mottle.

SUMMARY

A mosaic disease of Chinese cabbage (*Brassica pe-tsai*), prevalent in central California during the fall and winter months, is described.

The symptoms of the disease consist of a systemic clearing of the veins, followed by general mottling, with little or no distortion of the leaves.

In the greenhouse, transmission of the virus was obtained by mechanical inoculation with carborundum and also by means of the cabbage aphid (*Brevicoryne brassicae*) and the green peach aphid (*Myzus persicae*). Seed transmission tests gave negative results.

The virus was infectious after storage for 3 days at 22° C.; after a 10-minute treatment in a water bath at 73°; and after being diluted 1 to 5,000.

Local lesions were obtained on *Nicotiana glutinosa* and *N. tabacum*, but aside from these the host range was confined to the family Cruciferae.

Considered as to symptoms, the Chinese cabbage mosaic, cauliflower mosaic, and turnip mosaic viruses can easily be differentiated on Chinese cabbage, Winter Colma cabbage, and Purple Top White Globe turnip.

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EVALUATION OF SOME SOIL FUNGICIDES BY LABORATORY TESTS WITH PHYMATOTRICHUM OMNIVORUM[1]

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INTRODUCTION

Eradication of root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, by chemical treatment of the soil is attended by special difficulties. So far as is now known, *P. omnivorum* passes no essential portion of its life history above ground. It spreads from plant to plant by growing from root to root. Infected roots may be located from a few inches to 8 or more feet underground (*30*).[3] Therefore, to have any marked value in eradicating the fungus, a fungicide should be able to operate at much greater depths than materials used to treat diseases of seedlings or other diseases that are controlled at least in part by fungicidal treatment of the surface soil. Experience has in fact shown that materials commonly used as soil fungicides are not effective in eradicating *Phymatotrichum* root rot even from small experimental areas. This has necessitated consideration of other materials, including volatile materials capable of penetrating the soil. The present paper summarizes the laboratory phases of this work, which included development of methods for the evaluation of fungicides as to ability to permeate soil, as well as fungistatic and fungicidal effectiveness. Field trials of some of these materials are reported in an accompanying paper (*3*).

HISTORICAL REVIEW

Many workers with *Phymatotrichum* root rot have reported tests of fungicides. Pammel (*26*) reported field-plot tests of various chemicals. Heavy losses from root rot occurred despite the treatments. Curtis (*1*) attempted to control root rot on alfalfa by application of salt and kerosene.

King (*12, 13*) reported that further spread of root rot, in small areas in which the disease had just started, had been prevented by soaking them to a depth of 4 feet with a solution of 1 part of 40-percent formalin to 100 parts of water. Taubenhaus and Killough (*31*) found that 1 pint of formalin to 20 gallons of water, applied 2 gallons per square foot, did not control root rot. In further studies by King and Loomis (*15*), the edges of an active root rot area were treated with materials applied in irrigation water. Applications of 3

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[2] The writer is indebted to the late Dr. J. J. Taubenhaus, chief of the Division of Plant Pathology and Physiology, for advice in certain phases of this work; and to the Bayer-Semesan Co., Inc., and the Roessler & Hasslacher Chemical Co. (now R. & H. Chemicals Department, E. I. du Pont de Nemours & Co.), for supplying many of the chemicals tested.

[3] Reference is made by number (italic) to Literature Cited, p. 576.

acre-inches containing 1 percent of carbon disulphide did not greatly reduce root rot; but areas treated with a 2-percent solution of cresylic acid or a 1-percent solution of formaldehyde showed only a few dead plants late in the season. King, Loomis, and Hope (*16*) found that sclerotia of the fungus were inactivated by 30 minutes' exposure to solutions of more than 0.5 percent commercial formalin. Soil saturated with formaldehyde solutions inactivated sclerotia inserted therein; and the fungus was inactivated in sections of infected cotton roots exposed to the same soil within 4 days after the soil had been treated. Treatment of primary centers of infection with formalin was suggested by McNamara (*17*) as effective when tried early in the season. However, Dana (*4, 32*) reported unsuccessful results from formaldehyde treatments in field plots.

A large-scale test of formaldehyde in a 5-acre infested area near Indio, Calif., was made by the United States Department of Agriculture and the California Department of Agriculture. During May, June, and July, 1930, a 1.25-percent formalin solution was injected by pressure, at the rate of 1 gallon per cubic foot of soil, to a depth of 6 feet. The entire area was treated, with injections made 1 foot apart and 6 gallons of solution introduced at each point. Infected roots and root fragments removed from this treated area 10 months later were found by King and Hope (*14*) to be apparently dead. In 1932 (*20*), however, root rot recurred in certain places in this area. The infested areas were treated again; but the disease again recurred in 1933, in small areas toward the center of the treated region. In 1935 the disease again appeared in a very small spot, and this spot was again treated. Treatment of a second area, near Indian Wells, Calif., at the rate of 1 gallon per cubic foot of soil to a depth of 4 feet, was apparently more successful (*19*).[4]

Following the discovery of the sclerotial stage of the root rot fungus, Neal (*5*), King, Loomis, and Hope (*16*), and others have studied the toxic effect of various solutions on these bodies. King and Hope (*14*) found that fungicides effective against sclerotia or other separate portions of the fungus were not necessarily able to penetrate infected roots and kill the fungus inside such roots. Sclerotia installed inside small blocks made from the roots of plants and buried in soil were not killed by later treatment of the soil with formaldehyde.

Neal (*21*) and Neal, Wester, and Gunn (*24, 25*) have reported toxicity of ammonia, in solution or gaseous form, against mycelia as well as sclerotia of the root rot fungus, and have suggested the possible utilization of ammonia or ammonium compounds for its control. They stated that 6-percent solutions of ammonium hydroxide applied around large cotton plants in the field apparently killed the fungus in most cases, for when treated roots were removed and observed for 5 days in moist chambers, *Phymatotrichum* growth developed from only one of the eight roots. In 1934, Streets (*27*) stated that heavy applications of ammonium sulphate or ammonium hydrate, diluted to a safe concentration with water, around infected deciduous fruit and nut trees, yielded some promising results. More recently, Neal and Gilbert (*23*) have recommended dilute solutions (2 and 4 percent, respectively) of formaldehyde or ammonia water for eradication of root rot in small centers of infection or in areas

[4] Information partly from letters of Dr. D. G. Milbrath, dated April 25, 1934, and November 2, 1936.

to be planted to ornamentals or fruit trees. Neal and Collins (*22*) have found an initial concentration of about 1,000 p. p. m. of "N as NH_3" necessary to prevent growth from sclerotia in jars of soil.

Several notes on the development of the methods described in the present paper and on the preliminary results obtained have been presented elsewhere (*4*, *5*, *6*, *7*, *8*).

METHODS

The tests were chiefly of two types: (1) Toxicity tests that allowed the materials to act on the fungus without the interposition of soil, and (2) tests of the materials in moist soil to determine their ability to penetrate soil. These latter tests were run by various modifications of the soil-chamber or soil-culture technique. Jars or other containers were filled with unsterilized moist soil, inoculum of the fungus was added, and growth observed through the glass walls of the container. This direct observation is possible because the characteristic strands of *Phymatotrichum omnivorum* grow to a greater extent along the glass-soil surface than into the soil. Only rarely was there any doubt as to the identity of the fungus growth, and in such cases the containers were opened at the end of the experiment and identification checked by microscopic examination of the fungus.

In preliminary experiments set up in December 1928, mixtures of Uspulun, Semesan, and DuPont K–1–P with soil were placed in 1-liter Erlenmeyer flasks, and naturally infected cotton roots were used as inoculum. A more convenient container for the tests was suggested later by the work of Dana (*2*) and the soil-chamber tests described in this paper were run in mason jars instead of in flasks.

In most of the experiments, sclerotial masses were used as inoculum. These masses were taken in all cases from cultures of *Phymatotrichum omnivorum*, the writer's strain No. 24. Large masses produced on various synthetic media (*10*) were removed from the flasks, assembled on moist filter paper in moist chambers, and cut into cubes of approximately 2 or 3 mm. The inoculum used for each point in each jar of a soil-chamber experiment consisted of several portions from white, freshly produced sclerotial masses plus several portions from the older, buff or brown masses. The composite inoculum for every point of inoculation in each jar of a given experiment was thus made directly comparable to the inoculum used in every other jar of the experiment.

Growth starting from the sclerotial inoculum was observed through the glass, the precise extent being determined with a hand lens. Growth continued usually for about 5 to 7 days. Periodic examinations were made, usually after 7 and 14 days; and the longest growth (which was more uniform than the average radius of the colony) from each point of inoculation was noted, rather than the amount of growth within a set period. These maximum figures were averaged to obtain the "mean extent of growth" for each group of cultures as given in the tables.

Particular procedures followed are described below under the various experiments. For convenience, the results of each experiment are arranged with the more effective materials at the tops of the tables; this does not indicate the actual order of trial in the experiments.

SOIL-CHAMBER TESTS OF FUNGICIDES MIXED MECHANICALLY INTO THE SOIL

COMPARATIVE EFFECTIVENESS OF VARIOUS FUNGICIDES

Soil-chamber method A has served to determine for materials that are not too volatile the concentration required in moist soil to inhibit *Phymatotrichum* growth. Weighed or measured amounts of the materials to be tested (calculated as parts per million of the air-dry soil weight) were added to weighed quantities of air-dry Houston black clay surface soil, previously sifted through a ¼-inch mesh wire sieve. (This soil type, which is highly favorable for growth of the root rot fungus and occurs in regions in which the disease is very destructive, was used also in further experiments.) The fungicide was mixed thoroughly into the dry soil by repeated mixing. Sufficient distilled water was added to furnish a final soil moisture of 25 percent on an air-dry soil-weight basis, and incorporated into the soil by further mixing. The moist soil mixture was then distributed in 900-g portions into 1-quart mason jars, usually three for each concentration of each fungicide tested. The soil in the jars was tamped gently into place. Sclerotial inoculum was added the same day or within 2 or 3 days. Each jar was inoculated at two points, on opposite sides, with composite sclerotial inoculum. At each point the inoculum was inserted next to the glass wall of the jar, 2 to 4 cm below the upper surface of the soil mixture, and the soil firmed about it. The jars were covered with the regular mason jar lids, usually without the rubber rings.

The results of two typical experiments by this method are given in table 1. Copper carbonate was the least effective of the materials tested. Both K–1–X and No. 664 inhibited growth at 500 p. p. m. Mercuric chloride was highly fungistatic and prevented all growth even at the lowest concentration, 50 p. p. m.

TABLE 1.—*Comparison of various fungicides when mixed into soil, by method A, using 3 jars for each concentration of each material, and 6 check jars, each jar having been inoculated at 2 places*

Experiment and material tested	Mean extent of *Phymatotrichum* strand growth[1] from the inoculum, in jars with fungicides at concentration of—					
	50 p. p. m.	100 p. p. m.	250 p. p. m.	500 p. p. m.	1,000 p. p. m.	Check
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
Experiment C-4:						
Semesan (30 percent chlorophenol mercury)	41	20	Tr.	0	0	
K-1-X (2 percent ethyl mercury chloride)	31	26	17	Tr.	0	
No. 664 (14 percent nitrophenol mercury)	65	71	17	Tr.	0	
PMA (2.5 percent phenyl mercury acetate)	29	39	21	16	Tr.	
Copper carbonate	102	96	52	92	64	
None (check)						67
Experiment C-5:						
Mercuric chloride	0	0	0	0	0	
Bayer Special 100 × 3 (10 percent chlorophenol mercury)	36	8	Tr.	0	0	
Bayer Special II-6 × 5 (6 percent nitrophenol mercury)	27	15	Tr.	0	0	
K-1-X (2 percent ethyl mercury chloride)	14	18	13	Tr.	0	
Sterocide (4 percent mercury furfuramide)	10	19	16	Tr.	0	
Smuttox (4 percent oxymethylene)	25	20	40	25	33	
None (check)						35

[1] Growth indicated as Tr. consisted of very short, fuzzy growth on the inoculum, less than 10 mm long, and not penetrating soil particles near the inoculum.

INITIAL AND RESIDUAL EFFECTIVENESS OF ORGANIC MERCURY COMPOUNDS

The early laboratory results with the organic mercury compounds were used in planning field-plot tests of some of these materials for the control of root rot on cotton plants. The laboratory results indicated that K–1–X, for example, might be slightly more effective than No. 664 (table 1). In the field trials, however, applications of K–1–X failed to have any effect, while similar weights of No. 664 reduced the spread of root rot (*28*, *29*). The following experiment was planned to determine whether this apparent discrepancy between laboratory and field results might be explained by differences between the initial and the later effectiveness of the materials after contact with moist soil.

The experiment was set up by method A and growth from the inoculum was recorded as usual. After 3 weeks, however, the sclerotial inoculum was removed from the jars. The soil was made up to original weight with distilled water, mixed thoroughly, and placed in new jars. A second set of sclerotial inoculum, which was inserted in these new jars, was exposed to the residual inhibiting effect of the various fungicides which had now been in contact with the soil for 5 weeks. The results of both tests are summarized in table 2.

TABLE 2.—*Initial and residual efficiency of various fungicides when mixed into soil by method A, experiment C7, using 3 jars for each concentration of each material and 6 check jars, each jar having been inoculated at 2 places*

Material	Concentration in the soil	Mean extent of *Phymatotrichum* strand growth [1] in—	
		Initial test	Test after 5 weeks
	Parts per million	*Millimeters*	*Millimeters*
Semesan (DuBay) (30 percent chlorophenol mercury)	50	46	56
	100	39	49
	250	Tr.	0
	500	0	0
	1,000	0	0
DuBay 664 (14 percent nitrophenol mercury)	50	56	52
	100	48	63
	250	49	24
	500	0	0
	1,000	0	0
DuBay 965–D (2 percent ethyl mercury arsenate)	50	77	62
	100	67	36
	250	Tr.	48
	500	0	55
	1,000	Tr.	0
DuBay 971–A (2 percent ethyl mercury phosphate)	50	76	67
	100	63	53
	250	Tr.	41
	500	0	63
	1,000	0	0
K–1–X (2 percent ethyl mercury chloride)	50	77	74
	100	65	50
	250	34	91
	500	0	88
	1,000	0	90
PMA (2.5 percent phenyl mercury acetate)	50	90	79
	100	69	71
	250	43	60
	500	41	46
	1,000	23	23
Formaldehyde, U. S. P	50	108	52
	100	70	46
	250	90	43
	500	49	14
	1,000	77	52
	5,000	0	59
	10,000	0	0
Gasoline (commercial)	1,000	97	68
	5,000	0	66
	10,000	0	0
None (checks)		104	68

[1] See footnote 1, table 1.

In the first test, the effectiveness of the various materials in inhibiting growth of *Phymatotrichum* was much as in previous experiments. In the second test growth was inhibited at the same concentrations as originally in the Semesan and No. 664 mixtures only. The ethyl mercury compounds were much less effective than in the first tests. K–1–X, which in the first test had prevented *Phymatotrichum* growth completely at 500 p. p. m., in the final test did not impede growth at 1,000 p. p. m. Both 965–D and 971–A were effective at 250 p. p. m. in the initial test, but ineffective at 500 p. p. m. in the final test. Formaldehyde and gasoline also were markedly less effective in the final test.

In the initial test, as in previous tests of fungistatic effectiveness of materials shortly after mixture with soil, the ethyl mercury materials in general were much more effective than would be expected from their actual mercury content as compared to that of chlorophenol and nitrophenol mercury materials. But after exposure to the moist soil this high initial efficiency of the ethyl mercury materials had decreased; and the final, residual effectiveness correlated with the comparative concentration of mercury in the various materials. These results explained the amounts of the materials found to be effective in the first field-plot trials, and suggested more satisfactory rates of application for later trials with ethyl mercury compounds.

In several years of further field-plot trials with organic mercury compounds, repeated large additions of some of these materials greatly reduced the prevalence of root rot, but none was able to eradicate it (*28, 29*). Part of the difficulty with the mercurials was considered to be their failure to penetrate rapidly and deeply into the soil. In view also of the results reported at this time by Weiss and Evinger (*33*) in the use of naphthalene and alpha-naphthol against *Sclerotium rolfsii*, it seemed advisable to extend tests of possible soil fungicides for use against root rot to include some more highly volatile materials that might penetrate the soil more effectively. Many of these materials were not readily soluble in soil moisture and hence would not be fixed in solution in the particular soil layers in which they might be applied.

SOIL-CHAMBER TESTS OF THE EFFECTIVENESS OF VOLATILE MATERIALS IN PENETRATING SOIL AND PREVENTING GROWTH OF PHYMATOTRICHUM OMNIVORUM

A number of chlorinated hydrocarbons and other volatile materials were tested first by the following method, which indicated whether the materials could penetrate 2 to 4 cm through a standardized moist soil and then inhibit growth of the fungus from standardized sclerotial inoculum.

Soil-chamber method B differed from method A in that the materials to be tested were not mixed mechanically with the soil but were placed on the surface of the soil after the inoculum had been inserted in the jars. The quantity of material added was calculated as parts per million of the air-dry weight of soil in the jar. Liquid materials were poured rapidly directly on the soil surface; solid materials were placed in piles on the surface. The jar was then quickly sealed, using the rubber ring. Duplicate jars were used for each concentration of each material tested. Each jar was inoculated at two points, four sets of

inoculum thus being exposed to each concentration. Four jars were used for the checks in each series.

TABLE 3.—*Fungistatic effectiveness of various materials when placed on the surface of soil in soil chambers, in preventing growth from sclerotial inocula located 2 to 4 cm below, method B*

Experiment and material tested	Mean extent of *Phymatotrichum* strand growth from the inocula in jars where materials were applied at the rate of—				
	100 p. p. m.	500 p. p. m.	1,000 p. p. m.	2,000 p. p. m.	Check
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
Experiment C-8-a:					
Pentachlorethane	0	0	0	0	----------
Dichlorethylene	16	0	0	0	----------
Trichlorethylene	18	0	0	0	----------
Perchlorethylene	38	0	0	0	----------
Dichlormethane	70	40	0	0	----------
Acetone	58	59	49	91	----------
None (checks)	----------	----------	----------	----------	78
Experiment C-8-b:					
Xylene	0	0	0	0	----------
Carbon disulphide	0	0	0	0	----------
Benzene	19	0	0	0	----------
Kerosene saturated with paradichlorobenzene	30	0	0	0	----------
Ethylene dichloride	40	9	0	0	----------
Cresylic acid	56	25	17	19	----------
Sulphur chloride	81	75	85	71	----------
None (checks)	----------	----------	----------	----------	65
Experiment C-9:					
Turpentine	0	0	0	0	----------
Paradichlorobenzene	0	0	0	0	----------
Naphthalene	0	0	0	0	----------
Kerosene (commercial)	96	0	0	0	----------
Carbon tetrachloride	94	8	0	0	----------
Iodine	40	31	0	0	----------
Hexamethylenetetramine	41	69	58	31	----------
Thymol	55	31	41	43	----------
Alpha-naphthol	74	79	40	53	----------
Petroleum (lubricating) oil	58	55	74	63	----------
None (checks)	----------	----------	----------	----------	53

The results of three experiments by this method are listed in table 3. Pentachlorethane, xylene, carbon disulphide, turpentine, paradichlorobenzene, and napthalene completely inhibited growth from the sclerotial inoculum, even at 100 p. p. m. Five other materials allowed growth at this concentration but prevented it at higher concentrations. These materials seemed promising and were studied further, as described below.

EFFECTIVENESS OF VOLATILE MATERIALS AFTER PENETRATING DIFFERENT DEPTHS IN THE SOIL

Soil-chamber method C made it possible to determine the extent to which volatile materials would penetrate moist soil for more considerable distances, as shown by the effect on sclerotial inoculum in soil chambers. Method C was the same as method B, except that instead of using two sets of sclerotial inoculum toward the top of the jar, six sets were used, a pair toward the bottom, a pair toward the middle, and a pair toward the top. A diagram of this set-up is given in figure 1, *A*.

In filling a jar, two masses of inoculum were placed on the bottom at opposite sides. Half of the soil (by weight) for the jar was added and compacted to the usual density. Two more masses of inoculum were then placed on this soil and next to opposite walls of the jar. Most

of the rest of the soil was then added and compacted, the final two masses of inoculum inserted, and finally the remaining soil added and compacted. After this set-up was complete, the material to be tested was placed on the surface, and the jar lid sealed, using the rubber ring.

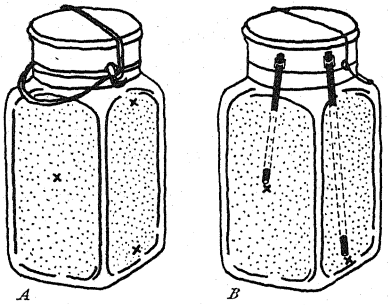


FIGURE 1.—Soil-chamber methods for testing penetration of fungicides through soil. *A*, Diagram of set-up by method C. Fungus inoculum was inserted in soil at points indicated by X marks as the jars were filled, and fungicides were then applied on surface of soil. *B*, Diagram of set-up by method D. Four glass tubes, capped with rubber tubing and glass rod, were installed in the soil. The fungicides were added, and after various periods the inoculum was pushed down the tubes to the points indicated, to determine residual fungistatic effectiveness.

TABLE 4.—*Fungistatic effectiveness of various materials, when placed on the surface of soil in soil chambers, in preventing growth from sclerotial inoculum located at different depths, method C*

Experiment and material tested	Rates of application [2]	Mean extent of *Phymatotrichum* strand growth,[1] from—		
		Upper inoculum	Middle inoculum	Bottom inoculum
	Parts per million	*Millimeters*	*Millimeters*	*Millimeters*
Experiment C-10:				
Pentachlorethane	100 to 2,000	0	0	0
Carbon disulphide	100 to 2,000	0	0	0
Turpentine	100 to 2,000	0	0	0
Paradichlorobenzene	100 to 2,000	0	0	0
Dichlormethane	100	33	38	40
	500	0	7	0
	1,000 and 2,000	0	0	0
Naphthalene flakes	100	0	11	12
	500	0	10	36
	1,000	0	5	24
	2,000	0	0	4
None (checks)		22	27	29
Experiment C-11:				
Tetrachlorethane	100 to 2,000	0	0	0
Xylene	100 to 2,000	0	0	0
Perchlorethylene	100 to 2,000	0	0	0
Trichlorethylene	100 to 2,000	0	0	0
Dichlorethylene	100 to 2,000	0	0	0
Benzene	100	18	30	26
	500 to 2,000	0	0	0
Kerosene	100	10	23	35
	500	0	0	8
	1,000	0	0	5
	2,000	0	0	0
None (checks)		33	40	39
Experiment C-14:				
Chloroform	100	55	50	56
	500 to 2,000	0	0	0
Ether	100	61	41	65
	500	66	54	58
	1,000	41	51	41
	2,000	Tr.	Tr.	Tr.
None (checks)		49	61	58

[1] See footnote 1, table 1.
[2] Materials in these experiments were all tested at 100, 500, 1,000, and 2,000 parts per million.

The upper inocula were at an average depth of about 15 mm below the surface of the soil, the middle inocula at about 75 mm, and the bottom inocula at about 134 mm. The various fungicides could prevent growth from the inoculum only after penetrating the soil to the depths mentioned. While such penetration through the moist soil materials in the jar would not prove that the fungicide would penetrate soil in the field, lack of penetration would strongly indicate the improbability of such penetration through soil under natural conditions.

TABLE 5.—*Fungistatic effectiveness of organic mercury compounds, formaldehyde and ammonia, as compared to other materials when placed on the surface of soil in soil chambers, in preventing growth from sclerotial inoculum located at different depths, method C*

Experiment and material tested	Rates of application	Mean extent of *Phymatotrichum* strand growth, from—		
		Upper inoculum	Middle inoculum	Bottom inoculum
	Parts per million	*Millimeters*	*Millimeters*	*Millimeters*
Experiment C–12:				
Pentachlorethane	100	0	0	0
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
Xylene	100	0	0	0
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
Paradichlorobenzene	100	1	0	3
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
DuBay 971–A (2 percent ethyl mercury phosphate).	100	28	28	40
	500	23	23	24
	1,000	15	10	14
	2,000	0	0	14
Semesan (30 percent chlorophenol mercury)	100	26	26	33
	500	36	38	45
	1,000	26	35	70
	2,000	0	29	54
Formaldehyde (U. S. P. 37 percent)	500	29	28	39
	1,000	26	30	39
	2,000	26	31	24
	4,000	14	26	36
Ammonia (NH_4OH, specific gravity, 0.90)	500	33	34	33
	2,000	1	49	43
	5,000	0	31	48
	10,000	0	3	40
None (checks)		50	44	53
Experiment C–15:				
Pentachlorethane	100	0	0	0
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
Ethyl chloride	100	53	56	53
	500	38	36	36
	1,000	0	0	0
	2,000	0	0	0
New Improved Ceresan (5 percent ethyl mercury phosphate).	100	45	63	60
	500	8	34	48
	1,000	0	29	61
	2,000	0	0	20
DuBay 1153–A (2.5 percent ethyl mercury phosphate).	100	40	50	50
	500	8	21	59
	1,000	0	31	51
	2,000	5	20	29
None (checks)		48	49	55

TABLE 6.—*Fungistatic effectiveness of smaller quantities of certain materials, when placed on the surface of soil in soil chambers, in preventing growth from sclerotial inoculum located at different depths, method C, experiment C–21*

Material tested	Rates of application	Mean extent of *Phymatotrichum* strand growth, from—		
		Upper inoculum	Middle inoculum	Bottom inoculum
	Parts per million	*Millimeters*	*Millimeters*	*Millimeters*
Industrial xylol	50	[1] 15	[1] 8	[1] 8
	75	0	0	0
	100	0	0	0
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
Tetrachlorethane	50	0	0	0
	75	[1] 16	[1] 21	[1] 23
	100	0	0	0
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
Industrial gasoline	50	0	0	0
	75	50	49	43
	100	40	30	31
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
None (checks)		41	41	39

[1] *P. omnivorum* growth in 1 of the 2 jars only.

Three experiments by this method are summarized in table 4. Nine of the materials were highly efficient, even at the lowest rate of application, inhibiting growth from even the bottom inoculum. Naphthalene prevented growth from the upper inoculum at 100 p. p. m., but was not completely effective against the deep inoculum even at 2,000 p. p. m. Ether penetrated the soil evenly but was able to inhibit *Phymatotrichum* development only when present at more than 1,000 p. p. m., while chloroform was effective at concentrations above 100 p. p. m.

In two experiments, C–12 and C–15 (table 5), organic mercury compounds, formaldehyde, and ammonia were compared with other materials. In experiment C–12, the Semesan and DuBay 971–A dusts were placed in appropriate amounts on the surface of the soil. More than half of the usual allotment of water for the soil of each jar had been reserved from the original mixing and was now added slowly over a period of 3 hours. The water trickled down fairly rapidly through the loose soil in the jars, and carried with it portions of the dusts. In experiment C–15, the New Improved Ceresan and DuBay 1153–A were merely placed on the moist surface soil, in the same way as the other materials, and the tops of the jars were sealed immediately. The ethyl chloride was handled in a flask cooled in a freezing mixture and dispensed rapidly with a pipette cooled in liquid ethyl chloride in the flask. The jars were sealed immediately after the ethyl chloride was placed on the surface of the soil; nevertheless, some must have escaped.

There were marked differences in the effectiveness of the various materials (table 5). Pentachlorethane and xylene, even at the lowest rates of application, completely inhibited growth of *Phymatotrichum* from the bottom inoculum. Paradichlorobenzene was not completely

effective at the lowest rate. Ethyl chloride penetrated the soil thoroughly but was effective only at concentrations above 500 p. p. m. All the other materials in experiments C–12 and C–15 were ineffective at the lower depths in the soil, although when present in relatively large amounts they inhibited growth from the upper inoculum. None of the four organic mercuries, irrespective of the method of application, inhibited growth from the bottom inoculum. The ethyl mercury-phosphate mixtures were more effective than formaldehyde in preventing growth from the upper inoculum.

Another experiment on the fungistatic effectiveness of certain materials was run by method C as in the experiments above, except that lower concentrations of the materials were tested. The results (table 6, experiment C–21), indicate that industrial gasoline is less effective against *Phymatotrichum* than is industrial xylol or tetrachlorethane. Xylol and tetrachlorethane were of approximately equal effectiveness. With each, 100 p. p. m. was uniformly effective in inhibiting growth of the fungus, while in a single jar receiving lower rates, there was definite although short growth from the inoculum. These results suggest that the rate of 100 p. p. m. of air-dry soil weight, used in most of the earlier tests, is probably near the minimum concentration required, even with the more effective of the fungicides tested, to inhibit growth of *P. omnivorum*.

It may be noted that the results obtained in these tests are not directly comparable to those obtained by other workers with other types of soil or with fungicides applied in dilute aqueous solution to drench the entire soil mass. In extensive studies on penetration of soil by fungicides, Hunt, O'Donnell, and Marshall (*11*) found that formaldehyde in dilute solutions percolated as rapidly as water through tubes of Manor and Leonardtown loams. Milbrath (*18*) similarly found that 1-percent solutions of formaldehyde passed through tubes of soil materials. Yet in the present work (table 5) undiluted United States Pharmacopoeia formaldehyde added on the surface of the heavy Houston soil in the jars, in sufficient quantity to furnish 4,000 p. p. m. of the air-dry weight of the soil, lowered but did not prohibit *Phymatotrichum* growth from inoculum located only 15 mm below the surface. The weight of formaldehyde added to furnish 4,000 p. p. m. was more than would be needed to furnish a 1-percent solution, as used by Milbrath, to saturate all the soil in the jar.

INITIAL AND RESIDUAL FUNGISTATIC EFFECTIVENESS OF PENTACHLORETHANE AND DUBAY 1153–A AFTER PENETRATING DIFFERENT DEPTHS IN SOIL

In the tests summarized above, pentachlorethane and several other chemicals were much more effective than the organic mercury materials. It was important to find whether this would be true also after the materials had been in contact with the moist soil for some time, and whether an organic mercury material might penetrate the soil by diffusing gradually downward.

An experiment was set up in which pentachlorethane and DuBay 1153–A were tested at rates ranging from 100 to 2,000 p. p. m. Pentachlorethane was placed on the surface of the soil, while the organic mercury dust was placed in a thin layer one-fourth to one-half inch below the surface of the moist soil. Two jars were used for

each rate of application of each material, in each of four series. Six jars for each series were used as checks. Series 1 and 2 were set up in soil chambers by method C, to determine the immediate fungistatic effectiveness of the materials.

Soil-chamber method D was used for series 3 and 4, to determine the residual fungistatic effectiveness. With this method the inoculum was not inserted until several weeks after the fungicides had been applied. As the jars were filled with soil, four glass tubes of 4-mm inside diameter were so placed that two reached nearly to the bottom of opposite walls of the jar, and the other two reached to the middle of alternate walls. Each tube was capped with a small piece of rubber tubing, which in turn was plugged with a bit of glass rod. The soil was carefully filled in and packed around these tubes, leaving the capped upper ends exposed (fig. 1, *B*). The fungicidal materials were applied at the beginning of the experiment just as in series 1 and 2. After 4 weeks for series 3, and 8 weeks for series 4, the jars were opened one by one, the glass plug on the upper end of each tube pulled out, and a mass of sclerotial inoculum pushed (by means of a slender glass rod) down through the tube and into contact with the soil. The glass rod was then withdrawn, the glass plug replaced to prevent later diffusion of the fungicide down the tubes, and the top of the jar replaced as soon as all four of the inocula had been inserted. No upper inoculum was used. This method allowed inocula to be placed deep in soil, already exposed to fungicides for considerable periods, without disturbing the soil and possibly allowing escape of volatile materials.

The jars of series 2 were opened and the soil surfaces exposed to gentle fanning for 5 hours on the second day and again on the third day after the beginning of the experiment. The effectiveness of the fungicides was evidently not affected by the short periods during which these jars were open; so series 1 and 2 both show initial effectiveness of the materials, and can be compared to series 3 and 4 representing effectiveness of the materials after 4 and 8 weeks, respectively.

As usual in soil-chamber tests, the pentachlorethane additions were completely effective in the lowest concentration tested against all six masses of inoculum in every jar, in series 1 and 2 (table 7). But after 4 or 8 weeks' contact with the soil the 100 p. p. m. concentration no longer exerted an inhibitive effect and 500 p. p. m. reduced but did not prevent growth. After 4 weeks the fungistatic effectiveness was only a fifth to a tenth of the original high value. Apparently an equilibrium was reached by this time, since the material was equally effective after an additional 4 weeks.

DuBay 1153–A caused only partial reduction in growth from the upper inoculum of those jars of series 1 and 2 that received 2,000 p. p. m., and was equally ineffective after 4 and 8 weeks. The ethyl mercury phosphate apparently did not diffuse downward sufficiently during the entire time to reduce fungus growth. But the aberrant results with this material in series 3 and 4 at 500 p. p. m. cannot be explained, and suggest the need of further test of the possibility of delayed penetration of soil by ethyl mercury phosphate.

TABLE 7.—*Initial and residual effectiveness of pentachlorethane and DuBay 1153–A (2.5 percent ethyl mercury phosphate) in preventing growth from sclerotial inoculum located at different depths, experiment C–16*

Series No. and method	Material tested	Rates of application	Mean extent of *Phymatotrichum* strand growth from—		
			Upper inoculum	Middle inoculum	Bottom inoculum
		Parts per million	*Millimeters*	*Millimeters*	*Millimeters*
1. Inoculum placed in jars as filled, jars not opened later.	Pentachlorethane	100	0	0	0
		500	0	0	0
		1,000	0	0	0
		2,000	0	0	0
	DuBay 1153–A	100	53	53	53
		500	48	46	55
		1,000	40	46	54
		2,000	5	41	51
	None (checks)		48	41	53
2. Inoculum placed in jars as filled, jars opened 5 hours each on third and fourth day.	Pentachlorethane	100	0	0	0
		500	0	0	0
		1,000	0	0	0
		2,000	0	0	0
	DuBay 1153–A	100	50	41	56
		500	44	44	63
		1,000	36	38	51
		2,000	8	31	61
	None (checks)		49	47	54
3. Inoculum inserted through tubes after 4 weeks.	Pentachlorethane	100		55	55
		500		16	20
		1,000		0	0
		2,000		0	0
	DuBay 1153–A	100		70	60
		500		0	0
		1,000		26	28
		2,000		43	59
	None (checks)			58	45
4. Inoculum inserted through tubes after 8 weeks.	Pentachlorethane	100		75	60
		500		9	8
		1,000		0	0
		2,000		0	0
	DuBay 1153–A	100		39	33
		500		0	0
		1,000		80	79
		2,000		81	69
	None (checks)			62	51

EFFECTIVENESS OF VARIOUS MATERIALS WITH INFECTED COTTON ROOTS USED AS INOCULUM

In the previous tests of volatile materials in soil chambers, portions of sclerotial masses of the fungus produced in artificial culture were used as inoculum. An experiment was planned to find whether the materials would be equally effective if infected roots were used instead. For this work cotton roots only partially decayed by root rot were collected on October 3, 1933. Since earlier work had shown that the fungus is most active near the advancing edge of the invaded portion of a root, each root was cut down to a piece 8 to 10 cm long, with most of the disintegrated lower portion removed and only about 3 cm of the upper uninvaded portion left above the infected area. Two roots were placed on the bottom of each mason jar, which was then filled with moist Houston soil. Six jars were used for each concentration of each material tested. Pentachlorethane, tetrachlorethane, and xylene were applied on the surface of the soil, at rates of 100, 1,000, and 2,000 p. p. m. of the air-dry soil weight.

Pentachlorethane prevented any *Phymatotrichum* growth from the infected roots. In both the tetrachlorethane and xylene series there was some strand development in one of the six jars receiving only

100 p. p. m. Profuse growth occurred in 9 of the 14 check jars with untreated soil. While pentachlorethane was apparently as effective in preventing growth from the infected roots as previously in preventing growth from sclerotial inoculum, tetrachlorethane and xylene were slightly less effective with the roots than with the sclerotial inoculum.

COMPACTNESS OF SOIL IN JARS AS AFFECTING PENETRATION AND FUNGISTATIC EFFECTIVENESS OF PENTACHLORETHANE

An experiment was set up by method C as outlined above, except for variations in the compactness of the soil. One series of soil chambers was prepared as usual with 900 g of moist Houston soil per 1-quart jar, another series with 1.2 times this weight, and a third with 1.4 times this weight. For the third series, the soil was pounded into the jars with pestles, compacting it to a bricklike mass. Pentachlorethane was added on the surface of the soil in amounts to furnish 100, 250, 500, and 1,000 p. p. m., respectively, of the air-dry weight of the 900 g of soil used in the usual set-up. The pentachlorethane inhibited growth from sclerotial inoculum at all three depths in every jar, while good growth occurred in the untreated jars of most of the series. In the check jars with the most compact soil, however, strands developed from only two of the four middle inocula, and from only one of the four bottom inocula. Absence of growth in pentachlorethane-treated jars of this set was therefore not so significant as in the other series.

In another experiment with soil compacted to 1.5 times the usual density, strand growth developed from one of the four middle inocula of the jars receiving 300 and 500 p. p. m. of pentachlorethane, and from some inocula at all depths in jars receiving 100 and 200 p. p. m.

In these tests, pentachlorethane readily permeated Houston soil somewhat more compact than that used in most of these laboratory tests, but did not penetrate uniformly through soil compacted to about 1.5 times this density.

APPLICATIONS OF VOLATILE FUNGICIDES IN CLOSED AS COMPARED TO OPEN SOIL CHAMBERS AND ON SURFACE OF SOIL AS COMPARED TO INSERTION IN HOLES

The several lines of experiment discussed above had shown that pentachlorethane, tetrachlorethane, and xylene, applied on the surface of soil in closed jars, even at very low concentrations, penetrated the soil and inhibited growth of *Phymatotrichum*. Obviously, conditions of this sort would not exist in the field, where the fungicides might readily evaporate and be lost in the air. Several experiments were therefore set up to determine the effectiveness of these volatile materials when used in open jars.

In the experiment summarized in table 8, each of the five series of jars included duplicate jars (set up with upper, middle, and bottom inoculum as described for method C above) for each of the four concentrations of pentachlorethane used. After pentachlorethane was added, the jars of series 1 were left open continuously; those of series 2 were closed for 2 hours; those of series 3 for 18 hours; and those of series 4 for 48 hours, and then left open afterwards. The jars of series 5 were closed continuously. Jars of series 1 to 4 were weighed at the beginning of the experiment and at intervals of 2 to 3 days thereafter, and water lost by evaporation was replaced.

TABLE 8.—*Fungistatic effectiveness of pentachlorethane in open as compared with closed jars, in preventing growth from sclerotial inoculum located at different depths, method C, experiment C–17*

Series No. and treatment of the jars	Rates of application	Mean extent of *Phymatotrichum* strand growth from —		
		Upper inoculum	Middle inoculum	Bottom inoculum
	Parts per million	*Millimeters*	*Millimeters*	*Millimeters*
1. Open continuously	100	68	56	56
	500	39	48	50
	1,000	0	0	0
	2,000	0	0	0
2. Closed for 2 hours, open thereafter	100	63	70	54
	500	0	28	66
	1,000	0	0	0
	2,000	0	0	0
3. Closed for 18 hours, open thereafter	100	35	33	56
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
4. Closed for 48 hours, open thereafter	100	0	0	40
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
5. Closed continuously	100	0	0	0
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0

Pentachlorethane was not so effective in the opened jars as in the series that remained closed (table 8). In the closed jars, pentachlorethane was completely effective, even at the lowest concentration, in preventing growth from the bottom inoculum. In the jars closed for 48 hours before being opened, growth occurred only from the bottom inoculum with pentachlorethane applied at 100 p. p. m. In the jars closed for only 18 hours, the 100-p. p. m. treatment did not inhibit growth from inoculum at any of the depths; and in the jars left open continuously or closed for only 2 hours before being opened, the 500-p. p. m. treatment was not effective.

Another experiment, summarized in part in table 9, was set up in 2-quart mason jars with 1,800 g of moist soil per jar. Pentachlorethane, tetrachlorethane, and xylene were tested, each in three separate series. In the first series, the fungicides were poured on the surface of the soil in the jars; in the second series the materials were poured into small holes, a half inch in diameter and 3 inches deep, punched in the center of each jar. The holes were filled in tightly with soil as soon as the fungicides had been inserted. These two series, and the untreated check jars, were left open and exposed to a constant breeze from an electric fan. At intervals of 2 to 3 days, each jar was weighed and the water lost by evaporation was replaced. There was apparently a fairly rapid loss of the fungicides from these jars. A strong odor was perceptible over the jars during the early days of the experiment. By the end of the first week, however, the odor was much weaker, and after 2 weeks it was imperceptible.

In a third series, not included in table 9, the jars were sealed as usual when method C was used. All three fungicides were effective in preventing growth from even the bottom inoculum, with the lowest concentration used, while profuse growth occurred in the jars of

untreated soil. Thus this series proved that the materials were able to penetrate the 165 mm of moist soil to the bottoms of these deeper, 2-quart jars.

TABLE 9.—*Relative fungistatic effectiveness of application of volatile materials on the surface of soil as compared to application in holes 3 inches deep in the soil, in open jars exposed to constant fanning, experiment C–20*

Methods of application	Material tested	Rates of application	Mean extent of *Phymatotrichum* growth from—		
			Upper inoculum	Middle inoculum	Bottom inoculum
		Parts per million	*Millimeters*	*Millimeters*	*Millimeters*
On surface of soil in jars	Pentachlorethane	100	24	54	80
		500	55	64	[1] [2] 34
		1,000	[2] 13	39	[1] 30
		2,000	0	[1] [2] 33	[1] 61
	Tetrachlorethane	100	34	58	71
		500	[2] 8	56	53
		1,000	24	86	59
		2,000	[1] [2] 15	58	104
	Xylene	100	41	66	59
		500	48	71	65
		1,000	[2] 18	74	58
		2,000	[1] [2] 11	69	70
In holes 3 inches deep in the soil	Pentachlorethane	100	[1] 38	49	76
		500	[1] 86	0	[1] [2] 28
		1,000	[1] 70	0	0
		2,000	[1] [2] 66	0	0
	Tetrachlorethane	100	39	58	64
		500	84	0	0
		1,000	[1] 134	0	0
		2,000	[1] [2] 23	0	0
	Xylene	100	80	74	84
		500	[1] 71	0	[1] [2] 16
		1,000	[1] 86	0	0
		2,000	[1] 61	0	0
None (checks)			49	57	68

[1] No growth recorded for first 7 days.
[2] Growth from only 1 or 2 of the 4 sclerotial masses.

The open jars exposed to constant fanning (table 9) yielded quite different results. None of the materials permanently inhibited fungus growth. Materials poured on the surface did not inhibit growth from the middle or bottom inoculum. Growth from the upper inoculum was inhibited only temporarily, except with pentachlorethane which held back growth in many jars for at least a week and permanently inhibited growth from the upper inoculum with 2,000 p. p. m. In the series in which the materials were inserted in holes 3 inches deep, on the other hand, the greater effect was observed on the deeper rather than on the upper inoculum. Growth was inhibited for the first week in almost all jars except those with the lowest concentrations of the fungicides. After this temporary effect, profuse growth was secured from most of the upper masses of inoculum. But the middle inoculum, which was nearest to the depth at which the fungicides had been inserted, did not grow; and there was growth from the deep inoculum only in one side of one jar at 500 p. p. m. of pentachlorethane, and from one side of a jar at 500 p. p. m. of xylene. During the second and third weeks, fungus strands from the upper inoculum spread down into the soil and around the middle and lower masses of inoculum, which still failed to grow and apparently were dead.

Since a concentration of only 100 p. p. m. of any of these fungicides was sufficient to inhibit growth of the fungus at any depth in the closed jars of this experiment, it is obvious that this concentration was not maintained in the open jars of either series. The repeated alternate wetting of the surface soil in these jars, followed by rapid drying from the fanning, resulted in frequent deep cracking of the soil and allowed unusual opportunity for rapid evaporation of the fungicides. This explains the results in the series in which the fungicides had been placed 3 inches deep in the soil. Early inhibition of growth from the upper inoculum here was presumably due to the presence, near the surface, of nontoxic concentrations of the materials, which were later lost by evaporation, and growth from the still viable inoculum then occurred. But deeper in the jars, higher concentrations must have been present long enough to kill the sclerotial masses before the fungicides were dissipated into the air.

This experiment made possible rather close comparison between pentachlorethane, tetrachlorethane, and xylene. Particularly in the first series with opened jars, pentachlorethane appeared slightly the most effective, both in inhibition of growth during the first week and in final prevention of growth. Tetrachlorethane and xylene appeared of almost exactly equal value.

These experiments suggest that the probable effectiveness of volatile fungicides in the field will be increased if, instead of the liquids being merely poured on the surface soil, they are inserted in holes deep enough to prevent too rapid loss from the surface, or confined by a cover of some sort for 18 to 48 hours. In preliminary field trials (*9*) volatile fungicides have accordingly been applied in holes.

TESTS OF THE TOXICITY OF VOLATILE MATERIALS

Most of the soil-chamber tests so far mentioned served to indicate only the fungistatic effectiveness of materials, that is, whether materials placed on top of the soil were able to prevent the fungus from growing. Further evidence of the probable value of materials as soil fungicides could be obtained by determining whether the materials were able to kill the fungus. Some toxicity tests were therefore run, separately from the soil-chamber studies and without considering the relative penetrating power of the materials in the soil.

In one experiment of this sort, bits of sclerotial masses from flask cultures of *Phymatotrichum omnivorum* were planted on the surface of agar slants. The tubes were sealed in 2-quart mason jars with the various materials to be tested. Sufficiently volatile materials could pass through the air and the cotton plugs in the tubes, and act on the fungus. The tubes were exposed to three arbitrary concentrations of materials: 0.144, 0.72, and 2.88 g per 2-quart jar. (These weights would provide concentrations respectively of 100, 500, and 2,000 p. p. m. if added to 1,800 g of moist Houston soil, sufficient to fill 2-quart jars. The concentrations were thus planned to be comparable to concentrations used in soil-chamber tests, but obviously only to the extent that moist soil in the jar in the soil-chamber test did not lower the effectiveness of a particular material by preventing it from diffusing, or increase effectiveness of a more pervasive material by filling part of the space with an immiscible volume of soil.) After exposure in the jars for periods respectively of 1, 7, and 14 days,

three tubes were removed for each concentration of each material, aerated for 1 hour in front of an electric fan, and incubated for several months to observe possible *Phymatotrichum* growth.

TABLE 10.—*Preliminary test of toxicity of volatile materials, confined in mason jars, to sclerotia of Phymatotrichum omnivorum exposed on agar slants for the periods indicated, experiment E–1*

Material tested	Growth (+) or lack of growth (−) of the fungus after exposure, for the number of days shown, to the indicated weights of material per 2-quart jars								
	0.144 g			0.72 g			2.88 g		
	1 day	7 days	14 days	1 day	7 days	14 days	1 day	7 days	14 days
Pentachlorethane	−	−	−	−	−	−	−	−	−
Ammonia	−	−	−	−	−	−	−	−	−
Xylene	[1] +	−	−	−	−	−	−	−	−
Carbon disulphide	[1] +	−	−	−	−	−	−	−	−
Formaldehyde	+	−	−	[1] +	−	−	[1] +	−	−
Paradichlorobenzene	+	+	+	+	[1] +	−	+	−	−
Turpentine	+	+	+	+	+	+	+	+	+
None (check)	+	+	+	+	+	+	+	+	+

[1] Growth in only 1 of 3 slants.

Marked differences were found (table 10) between the various materials. Pentachlorethane and ammonia prevented any further growth of the fungus, even at the lowest concentration and for the shortest period of exposure; while paradichlorobenzene was only slightly toxic and turpentine did not prevent growth even during the period of treatment.

In the series just described, weakly fungicidal materials absorbed in the agar substratum might possibly have prevented later growth even from uninjured inocula. The exposure to carbon disulphide, for example, had discolored the agar; and the ammonia had produced a fine precipitate in the agar.

Later toxicity tests were accordingly run by a modified procedure. In this procedure, bits of sclerotial masses of *Phymatotrichum omnivorum*, resting on small wads of moist absorbent cotton in culture tubes plugged with nonabsorbent cotton, were exposed to the fumes of volatile materials confined within 2-quart jars. After specified intervals, the tubes were removed from the jars, aerated in front of an electric fan for 1 hour, and each sclerotial piece was then transferred aseptically to an agar slant. The fungicide itself could be carried over to the agar slant only by passing through the air and the cotton plug and depositing on or in the bit of inoculum. The entire procedure was carried out aseptically, so far as the inoculum was concerned, so that no contaminations entered and observations on large numbers of cultures could be made rapidly and with little trouble. There was no necessity for aseptic precautions with regard to the mason jar containing the fungicide.

In the following experiments the culture tubes used inside the jars each contained a wad of about 0.2 g of absorbent cotton, moistened with 3 cc of distilled water. These tubes were autoclaved, then a piece about 2 by 1 by 1 mm of a fresh, white sclerotial mass of *Phymatotrichum omnivorum* was deposited aseptically in each tube. Three or

more tubes, for each harvest of each concentration of each material to be tested, were placed in 2-quart mason jars. The material to be tested was then introduced into the jar, to the side of the tubes, care being taken to prevent contact with the cotton plugs. Immediately afterward the jar was sealed tightly and tilted just enough to facilitate entrance of heavy gases into the tubes, but not enough to allow the liquid or solid material to come in contact with the plugs. After the respective periods, the jar was opened and the tubes removed quickly. The sclerotial masses were transferred to slants of synthetic medium No. 135 (*10*). The slants were incubated for 3 to 4 months and examined periodically.

To test the possibility that the period of observation in these toxicity tests might not be long enough, certain slants were held longer, in some cases for more than 8 months. In one series, the inoculum was transferred at approximately monthly intervals from the old agar slants to fresh slants, to dispose of traces of fungistatic substances. Another set of sclerotial masses, of which some had been treated with pentachlorethane and others with paradichlorobenzene, were taken from the agar slants on which they had failed to grow, sliced into smaller pieces to expose fresh surfaces, and distributed to new agar slants. None of these special treatments resulted in growth from inocula that had not previously produced growth. Those that failed to grow within the usual period of incubation probably were dead.

In experiments E–2, E–3, and E–4, a single jar was used for the three periods of exposure to a particular concentration of a material. Thus in experiment E–2, nine tubes were placed in the jar with the material; the jar was opened after 24 hours, three tubes withdrawn, and the jar quickly sealed again; it was opened 48 hours later for the second harvest; and after 7 days for the final harvest. Some of the volatile material was lost each time the jar was opened, even though it was closed again within 7 or 8 seconds. This loss lowered progressively the concentration of materials in the jars with the lowest rates, but did not affect results with the higher rates since the materials were present usually in such excess that liquid or solid material remained unvolatilized on the bottom of each jar at the end of the experiment.

These three experiments, summarized in table 11, showed sharp differences in the ability of the various materials placed in the mason jars to pass through the air and through the cotton plugs and then kill the bits of *Phymatotrichum* sclerotial masses. Pentachlorethane, tetrachlorethane, xylene, and ammonia were completely toxic, at the lowest concentration tested, and with a period of exposure of only 24 hours. Carbon disulphide and a considerable group of other materials were somewhat less effective, and naphthalene and alpha-naphthol were completely ineffective. The two organic mercury preparations included in the tests were ineffective even in the highest concentration with only 1 day of exposure, but toxic to the lowest concentration after 4 days' exposure.

To obviate uncertainty of concentration of materials present in the jars after the first period of exposure, a series of experiments E–5, E–7, and E–8 was run in which a separate mason jar was used as the container for each set of tubes for each harvest. In experiment E–5, ethyl chloride, pentachlorethane, and DuBay 1153–A were included,

the same three weights of materials being used as in the previous experiments. The ethyl chloride was measured as a liquid from a flask cooled in a freezing mixture, and sealed in the jars with little loss. It was not toxic even at the highest concentration after 7 days (table 12). Pentachlorethane killed all the inoculum, at every concentration, within 24 hours. DuBay 1153–A was again completely ineffective even at the highest concentration with only 24 hours' exposure, but prevented all growth from sclerotia that had been exposed to even the lower concentration for 3 or for 7 days.

TABLE 11.—*Toxicity of materials confined in mason jars to sclerotia of Phymatotrichum omnivorum exposed on wads of moist cotton for different periods and then transferred to agar slants*

Experiment number and material tested	Growth (+) or lack of growth (−) of the fungus after exposure for time shown to the indicated weights of material per 2-quart jars								
	0.144 g			0.72 g			2.88 g		
	1 day	3 days	7 days	1 day	3 days	7 days	1 day	3 days	7 days
Experiment E–2:									
Pentachlorethane	−	−	−	−	−	−	−	−	−
Xylene	−	−	−	−	−	−	−	−	−
Ammonia	−	−	−	−	−	−	−	−	−
Carbon disulphide	+	[1]+	−	−	−	−	−	−	−
Perchlorethylene	+	+	+	−	−	−	−	−	−
Formaldehyde	+	+	−	+	−	−	[1]+	−	−
Paradichlorobenzene	+	+	−	+	−	−	+	−	−
None (checks)	+	+	+	+	+	+	+	+	+
Experiment E–3:									
Tetrachlorethane	−	−	−	−	−	−	−	−	−
Trichlorethylene	+	+	+	−	−	−	−	−	−
Dichlorethylene	+	+	+	−	−	−	−	−	−
Carbon tetrachloride	+	+	+	−	−	−	−	−	−
Dichlormethane	+	+	+	+	[1]+	[1]+	−	−	−
Benzene	+	+	+	+	+	+	+	+	−
Naphthalene	+	+	+	+	+	+	+	+	+
None (checks)	+	+	+	+	+	+	+	+	+
	1 day	4 days	7 days	1 day	4 days	7 days	1 day	4 days	7 days
Experiment E–4:									
Chloroform	+	+	[1]+	−	−	−	−	−	−
Ethylene dichloride	+	+	+	−	−	−	−	−	−
Ether	+	+	+	+	+	+	−	−	−
New Improved Ceresan	+	−	−	+	−	−	+	−	−
DuBay 1153–A	+	−	−	+	−	−	+	−	−
Acetone	+	+	+	+	+	+	[1]+	−	−
Alpha-naphthol	+	+	+	+	+	+	+	+	+
None (checks)	+	+	+	+	+	+	+	+	+

[1] Growth from only 1 of 3 slants.

Experiment E–7 was run with pentachlorethane and hexachlorethane, each at additional concentrations of 0.072 and 0.36 g per 2-quart jar. (On the theoretical comparative basis of the weight required as additions to sufficient Houston soil to fill the jars, these would correspond to additions of 50 and 250 p. p. m. of air-dry soil weight.) Pentachlorethane killed all the inocula exposed to any of these concentrations for either 16, 22, or 40 hours. Hexachlorethane, on the other hand, was not toxic at any of the concentrations, after even the longest periods. This solid, most highly chlorinated of the ethane series is thus apparently of no value as a fungicide against *Phymatotrichum omnivorum*.

TABLE 12.—*Toxicity of volatile materials confined in mason jars to sclerotia of Phymatotrichum omnivorum exposed on wads of moist cotton for the periods indicated and then transferred to agar slants, quadruplicate tubes with separate mason jar being used for each period of exposure with each concentration of each material*

Experiment No. and material tested	Periods of exposure	Growth (+) or lack of growth (−) of the fungus after exposure to the indicated weights of material per 2-quart jar				
		0.072 g	0.144 g	0.36 g	0.72 g	2.88 g
Experiment E–5:						
Pentachlorethane	1 day		−		−	−
	3 days		−		−	−
	7 days		−		−	−
DuBay 1153–A	1 day		+		+	+
	3 days		−		−	−
	7 days		−		−	−
Ethyl chloride	1 day		+		+	+
	3 days		+		+	+
	7 days		+		+	+
None (checks)	1 day		+		+	+
	3 days		+		+	+
	7 days		+		+	+
Experiment E–7:						
Pentachlorethane	16 hours	−	−	−	−	
	22 hours	−	−	−	−	
	40 hours	−	−	−	−	
Hexachlorethane	16 hours	+	+	+	+	
	22 hours	+	+	+	+	
	40 hours	+	+	+	+	
None (checks)	16 hours	+	+	+	+	
	22 hours	+	+	+	+	
	40 hours	+	+	+	+	
Experiment E–8:						
Tetrachlorethane	16 hours	−	−	−	−	
	24 hours	−	−	−	−	
	3 days	−	−	−	−	
Xylol (industrial)	16 hours	+	−	−	−	
	24 hours	+	−	−	−	
	3 days	+	−	−	−	
Gasoline (industrial)	16 hours	+	+		+	+
	24 hours	+	+		+	[1] Tr.
	3 days	+	+		−	−
	7 days	+	+		−	−
None (checks)	16 hours	+	+	+	+	+
	24 hours	+	+	+	+	+
	3 days	+	+	+	+	+
	7 days	+	+	+	+	+

[1] Growth from only 1 slant.

Experiment E–8 (table 12) was a comparison of tetrachlorethane with industrial xylol and industrial gasoline. In the series were included the short period of exposure and low rate of application as used in the previous experiment. Tetrachlorethane was completely effective in this stringent test, while xylol was not effective at the lowest rate with any of the periods of exposure and gasoline was effective only after 3 days exposure to the higher rates. Use in these experiments of the shorter period of exposure and lower rates permitted differentiation of pentachlorethane and tetrachlorethane as slightly more toxic than xylol under these conditions.

COMPARISON OF TOXICITY RATINGS WITH THE FUNGISTATIC EFFECTIVENESS OF MATERIALS AFTER PENETRATING THE SOIL

In the experiments recorded above some possibly fungicidal materials have been considered chiefly from two aspects. The studies in soil chambers (tables 1 to 9) were essentially fungistatic tests, designed

primarily to determine the ability of the materials at various concentrations to penetrate moist soil and there to inhibit growth of the root rot fungus. In the toxicity tests (tables 10 to 12), the materials were judged by another criterion, namely, their ability to volatilize through air and through dry cotton plugs and then kill or permanently inactivate small portions of *Phymatotrichum* sclerotial masses. Obviously, materials highly effective by both tests are more promising as possible fungicides than materials that proved to be effective by one but not by both tests. It is thus of interest to compare the results in summarized form, as in table 13.

TABLE 13.—*Comparative effectiveness of materials in killing sclerotial masses of Phymatotrichum omnivorum exposed only to fumes of the materials, and in penetrating moist soil to inhibit growth from sclerotial masses*

[Arranged with the more effective materials at the top]

Toxicity rating [1]		Fungistatic rating [2]	
Conditions	Material	Rates of application	Material
		Parts per million	
1 day, at low concentration.	Pentachlorethane.		Pentachlorethane.
	Tetrachlorethane.		Tetrachlorethane.
	Xylene.		Xylene.
	Ammonia.		Carbon disulphide.
1 day at medium or 4 days at low concentration.	Carbon disulphide.	100	Perchlorethylene.
	Perchlorethylene.		Trichlorethylene.
	Trichlorethylene.		Dichlorethylene.
	Dichlorethylene.		Turpentine.
	Carbon tetrachloride.		Paradichlorobenzene.
	Chloroform.		Chloroform.
	Ethylene dichloride.	500	Dichlormethane.
	New Improved Ceresan.		Benzene.
	DuBay 1153-A.		Gasoline.
1 day at high or 7 days at low concentration.	Formaldehyde.	1,000	Kerosene.
	Paradichlorobenzene.		Ethyl chloride.
	Dichlormethane.	2,000	Ether.
	Ether.		Naphthalene.
3, 4, or 7 days at high concentration.	Gasoline.		New Improved Ceresan.
	Benzene.		DuBay 1153-A.
	Acetone.		DuBay 971-A.
	Ethyl chloride.		Semesan.
Not toxic in limits of tests.	Hexachlorethane.	[3] 2,000	Acetone.
	Naphthalene.		Cresylic acid.
	Turpentine.		Sulphur chloride.
	Alpha-naphthol.		Hexamethylenetetramine.
			Alpha-naphthol.
			Petroleum (lubricating) oil.
		[3] 4,000	Formaldehyde.
		[3] 10,000	Ammonia.

[1] Volatilizes through air and cotton plugs, and kills sclerotial masses in time indicated.
[2] Penetrates 135 mm of moist soil and inhibits growth when applied in soil chambers at rates indicated.
[3] Not effective at concentration given.

Three of the materials, pentachlorethane, tetrachlorethane, and xylene, were completely effective in low concentrations by both criteria. Another group of materials, including carbon disulphide, perchlorethylene, trichlorethylene, and dichlorethylene, were effective at 100 p. p. m. in the test of fungistatic effect, but fell into the second group in the toxicity test. These materials are, therefore, apparently not quite so likely to be effective under soil conditions as the first three. Paradichlorobenzene, which belongs in the first group on the basis of its ability to penetrate soil and inhibit growth, is shown by the toxicity test to be rather slowly toxic.

Some of the other materials appeared more promising from the standpoint of toxicity than from that of ability to penetrate the soil and inhibit growth. Thus New Improved Ceresan and DuBay 1153–A were fairly toxic but were not able to penetrate the soil to any practical extent. Ammonia was very toxic but did not penetrate the soil effectively even when added at 10,000 p. p. m., the highest rate tested for any of the materials. Formaldehyde was moderately toxic, but of no apparent value when judged by its ability to penetrate moist Houston black clay soil.

Laboratory study of a variety of materials by these several tests has furnished rather critical data for determining their probable value as fungicides against *Phymatotrichum* root rot. While the tests are completely arbitrary, materials that fail to be effective in these laboratory tests will probably be less useful under field conditions than materials that are effective in the tests. The results indicate that the general failure of some fungicides to eradicate *Phymatotrichum* root rot is due probably to their low effectiveness in permeating the soil.

On the basis of the results obtained, pentachlorethane, tetrachlorethane, and xylene appear to be particularly worthy of field trial against root rot. Obviously, however, none of these materials should be recommended for practical use without thorough trial in field experiments. In field trials against *Phymatotrichum* root rot, pentachlorethane, tetrachlorethane, and xylol applied around infected plants have regularly killed the fungus in the roots of the plants. As yet, however, the methods tried for applying these materials in the field have not succeeded in completely eradicating the disease from infested areas. These field experiments are summarized in an accompanying paper (*3*). None of the soil fungicides mentioned in the present paper are recommended at this time for practical use against *Phymatotrichum* root rot.

SUMMARY

Methods were developed for evaluating fungicides in the laboratory as to ability to permeate soil and as to fungistatic and fungicidal effectiveness against the root rot fungus, *Phymatotrichum omnivorum*.

Tests with fungicides mixed mechanically into soil indicated the relative effectiveness of a number of organic mercury compounds for preventing growth from inoculum of *P. omnivorum*. The initial fungistatic effectiveness of ethyl mercury materials was found to be much higher than would be expected from their mercury content, but the residual value after 5 weeks' contact with moist soil was in line with that of other compounds.

Tests with fungicides applied on the surface of Houston black clay soil in closed jars showed whether materials were able to penetrate moist soil in jars, to various depths, and then prevent growth of *P. omnivorum*. A group of volatile materials, including pentachlorethane, tetrachlorethane, xylene, carbon disulphide, turpentine, perchlorethylene, trichlorethylene, and dichlorethylene, applied at only 100 p. p. m. of the air-dry soil weight, completely inhibited growth from inoculum 135 mm deep in the soil. Formaldehyde, on the other hand, was ineffective even at 4,000 p. p. m.; and ammonia did not prevent growth from deep inoculum even at 10,000 p. p. m. The organic mercury compounds tested prevented growth from inoculum near the surface but not from deeper inoculum.

By using a method in which the inoculum was inserted deep in soil in closed jars several weeks after pentachlorethane had been applied on the surface, it was found that 4 to 8 weeks' contact with moist soil reduced the fungistatic effectiveness to between a fifth and a tenth of the original value.

Pentachlorethane, tetrachlorethane, and xylene when applied at only 100 p. p. m. on the surface of Houston soil in closed jars were quite effective in preventing growth from *P. omnivorum* inoculum buried in the soil, but these low concentrations were not regularly effective under more adverse conditions, as with very compact soil, or with infected roots as the inoculum, or in experiments in which the jars were left open and fanned to accelerate surface loss of volatile fungicides. Higher concentrations—500 to 1,000 p. p. m.—appeared necessary under these conditions. In open jars, there appeared considerable advantage in inserting the fungicide in holes below the surface rather than applying it on the surface; yet in one experiment the inoculum located just below the surface of the soil survived such treatment.

Actual toxicity of the volatile materials was determined by a method that required the fungicides to volatilize through air and through cotton plugs before coming in contact with the fungus inoculum. Pentachlorethane, tetrachlorethane, xylene, and ammonia proved most toxic to *P. omnivorum* in these tests.

Pentachlorethane, tetrachlorethane, and xylene showed ability to penetrate moist soil and prevent growth of *P. omnivorum*, together with high fungicidal value after passage through air, and these materials are suggested as promising soil fungicides for trial in field experiments. None of the soil fungicides mentioned in this paper are recommended at this time for practical use against *Phymatotrichum* root rot.

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TESTS WITH PENTACHLORETHANE, TETRACHLORETHANE, AND XYLOL TO DETERMINE THEIR EFFICIENCY IN ERADICATION OF PHYMATOTRICHUM ROOT ROT[1]

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INTRODUCTION

An accompanying paper (*1*)[2] summarizes previous studies on possible control by soil fungicides of root rot caused by *Phymatotrichum omnivorum* (Shear) Duggar, and presents results of laboratory comparisons of chemicals for their relative value as soil fungicides against *P. omnivorum*. These experiments demonstrated that most of the water-soluble materials tested, such as formaldehyde, ammonia, and organic mercury compounds, were apparently unable to penetrate rapidly through moist Houston black clay soil under the conditions of the tests. A number of other materials, including pentachlorethane, tetrachlorethane, and xylene, mostly not water-soluble, readily permeated the soil. Separate comparisons of the relative toxicity of chemicals to *P. omnivorum*, under conditions that required passage through air and dry cotton plugs, indicated high toxicity values also for pentachlorethane, tetrachlorethane, and xylene. These results suggested the desirability of field trial of the three chemicals for possible use against *Phymatotrichum* root rot. The present paper summarizes the trials made with these materials between September 1933, and October 1935.

MATERIALS AND METHODS

The materials used were commercial refined pentachlorethane,[3] specific gravity 1.685, commercial refined tetrachlorethane, specific gravity 1.58, and industrial xylol (xylene), specific gravity 0.86. Some experiments included also a partially refined tetrachlorethane, referred to below as "crude" tetrachlorethane. This was a somewhat turbid, dark greenish-yellow liquid with a pungent odor.

The experiments were carried out at College Station and Bryan, Tex., with various plants growing in Lufkin fine sandy loam soil. This soil has a highly plastic and impervious clay subsoil; nevertheless the roots of cotton and other plants penetrate it deeply.

The experiments were of three distinct types, and the special methods used will be presented separately. However, the fungicides were applied in each case in holes pierced with a crowbar to a depth of 6 inches. One such hole was used for each 6 inches square of the area to be treated, or four holes to each square foot. The holes were located by measurement uniformly over treated areas.

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[2] Reference is made by number (italic) to Literature Cited, p. 593.

[3] The pentachlorethane and tetrachlorethane used in these experiments were furnished through the courtesy of the R. & H. Chemicals Department, E. I. du Pont de Nemours & Co.

The laboratory results (*1*) had indicated that while fungicide concentrations of 100 parts per million of the soil weight were effective in closed jars, concentrations of 500 to 1,000 parts per million would probably be necessary for effective use under more or less adverse conditions. Accordingly, the following rates were calculated:

Rates of application, as grams per square foot	*Equivalent to concentrations as parts per million of soil weight to a depth of 4 feet*
249	1,500
166	1,000
83	500
41.5	250

It should be emphasized that while the theoretical depth of 4 feet was arbitrarily used in calculating rates of application, the fungicides were actually applied only in the holes 6 inches deep, and not to the depth mentioned. Weights of fungicides for each hole were calculated to volume. These volumes were measured in the field for each hole, the material was poured in, and the soil pushed into the top of the hole. Each hole was covered in this way with soil rapidly enough to avoid appreciable evaporation of the volatile materials from the open holes; but the materials were found to evaporate later through the soil. Odors of the various fungicides were noticed over treated plots for several days or even weeks after the materials had been applied.

The first field trials of these volatile fungicides were made by applying them in relatively small areas surrounding plants recently attacked by root rot, and then after various periods digging out the roots of the plants and determining the viability of the fungus on and within the roots. The roots were obtained by digging a trench near the plants, and then separating the roots of each plant from the soil by carefully picking the remaining soil away with ice picks. The roots were cut at once into sections corresponding to the depths in the soil, and placed in mason jars containing a little water. These jars were taken into the laboratory, the excess water was poured out, and Houston black clay surface soil containing 25 percent moisture on an air-dry soil-weight basis was filled in around the roots. Soil cultures prepared in this way are a more reliable means of demonstrating the viability of *Phymatotrichum omnivorum* on diseased roots than are cultures prepared aseptically on culture media (*6, p. 771*). The fungus if alive grows out from the root and develops in the soil-glass interface where it is readily observed. In the present experiments, soil cultures were observed usually for 4 to 6 weeks, and doubtful growth was always examined under the microscope.

Absence of growth in soil cultures made in this way from treated roots was taken to indicate that the fungus was not viable. However, two possible sources of error should be noted. (1) A root from a treated area might carry with it fungicide taken up from the soil and inhibit growth from fungus material not actually killed. (2) An area selected for experimental treatment might be one in which plants had been attacked by the disease some time before. As has been pointed out (*6, pp. 768–773*), *Phymatotrichum omnivorum* has never been recovered by the writer or his associates from thoroughly decayed roots of plants that have succumbed to the disease, and failure to obtain the fungus from fungicide-treated roots of this kind could not be considered evidence that the fungicide had killed the fungus. To obviate this

difficulty, these experiments were invariably carried out with plants only recently attacked, on the roots of which the fungus would still be advancing actively, and untreated, check areas were always selected with the disease in comparable stages.

RESULTS OF APPLYING THE MATERIALS AROUND DISEASED PLANTS

APPLICATION OF TETRACHLORETHANE AROUND COTTON PLANTS IN 1933

A preliminary field test with tetrachlorethane was run in a cotton (*Gossypium hirsutum* L.) field at College Station during the fall of 1933. Ten comparable areas were selected, on September 21, along the advancing margins of root rot spots. Tetrachlorethane was applied in areas of 12 square feet, the treated areas extending 4 feet along the row and 1½ feet to each side. Each area included several plants that had just succumbed to root rot and several others that as yet showed no signs of the disease above ground, but were presumably already infected. Three areas in different parts of the field were marked off and treated at 166 g and three at 83 g per square foot, and four additional areas were held as checks.

Close observation during the experiment showed no definite injury to the treated plants that could be attributed to the tetrachlorethane. Plants in adjoining rows, only 1½ feet from the treated areas, showed no injury from the tetrachlorethane during the several months before frost.

The effect of the treatment on the root rot fungus was determined, after 15, 21, and 32 days, by excavating sample areas of each rate of application and the checks. Trenches 2 feet deep were dug next to the plants, and the roots were separated and placed in soil cultures for testing as described above. The results are summarized in table 1.

TABLE 1.—*Effects of tetrachlorethane applications at different rates around cotton plants with root rot, September 1933*

Rate of application per square foot (grams)	Interval before excavation of roots	Plants in plot	Results of testing portions of roots in jars of moist soil		
			Portions tested	Portions yielding *Phymatotrichum omnivorum*	
	Days	*Number*	*Number*	*Number*	*Percent*
166	15	8	20	0	0
	21	5	15	0	0
	32	5	15	0	0
83	15	7	18	1	6
	21	7	21	0	0
	32	7	23	0	0
0 (checks)	15	7	25	15	60
	21	4	15	4	27
	32	6	14	2	14
	32	5	12	1	8

Profuse *Phymatotrichum* strand growth developed from the roots of at least one plant from each check area. From the 39 cotton plants from the treated areas, on the contrary, there was growth only from the 12- to 18-inch deep portion of one taproot, in exceptionally dense clay, from a plot treated at the lower rate and dug after 15 days. As

will be noted in figure 1, this root was located near the edge of the treated area.

Figure 1 shows diagrammatically the results obtained from the

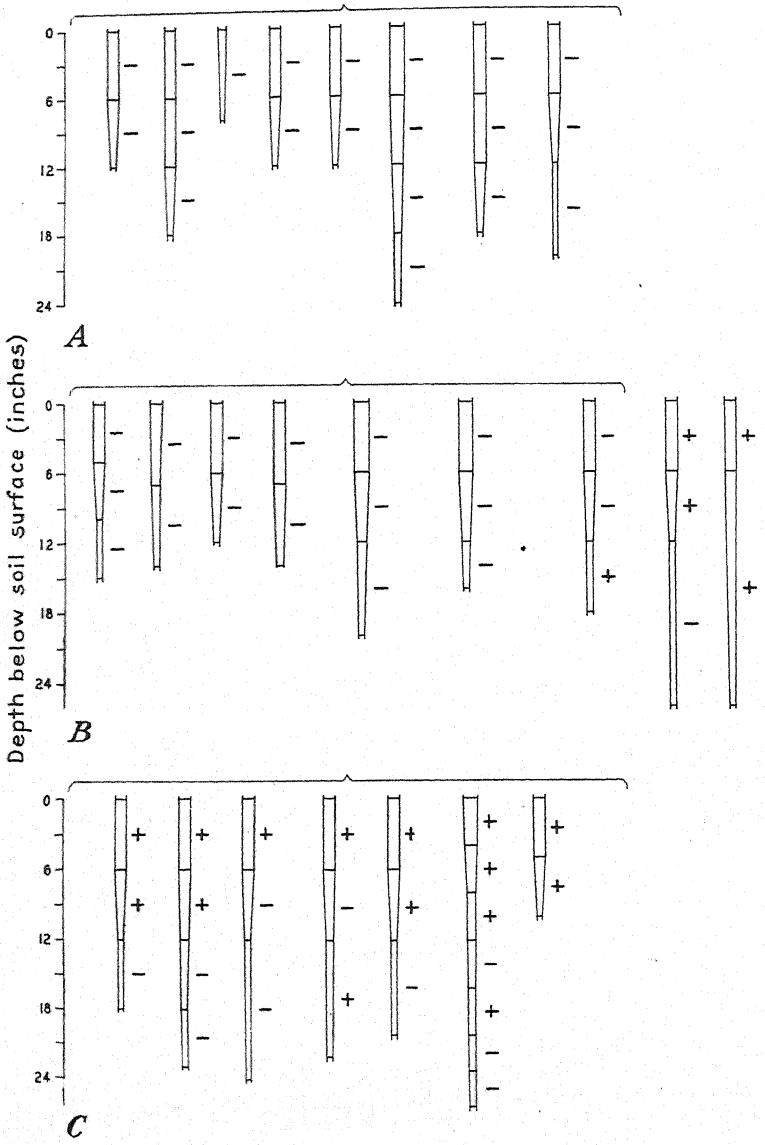


FIGURE 1.—Diagrammatic summary of results in plots excavated 15 days after tetrachlorethane was applied in areas designated by braces. Cotton plants were located within or near the plots at approximate positions shown. Roots were cut into portions as indicated, later growth of *Phymatotrichum* strands from these portions in soil cultures being indicated by + and lack of growth by −: *A*, Application at rate of 166 g per square foot; *B*, application at rate of 83 g per square foot; *C*, not treated, check.

three plots excavated 15 days after tetrachlorethane had been applied. It will be noted that the root rot fungus had been killed by this time on roots from the treated area, with the exception mentioned, but

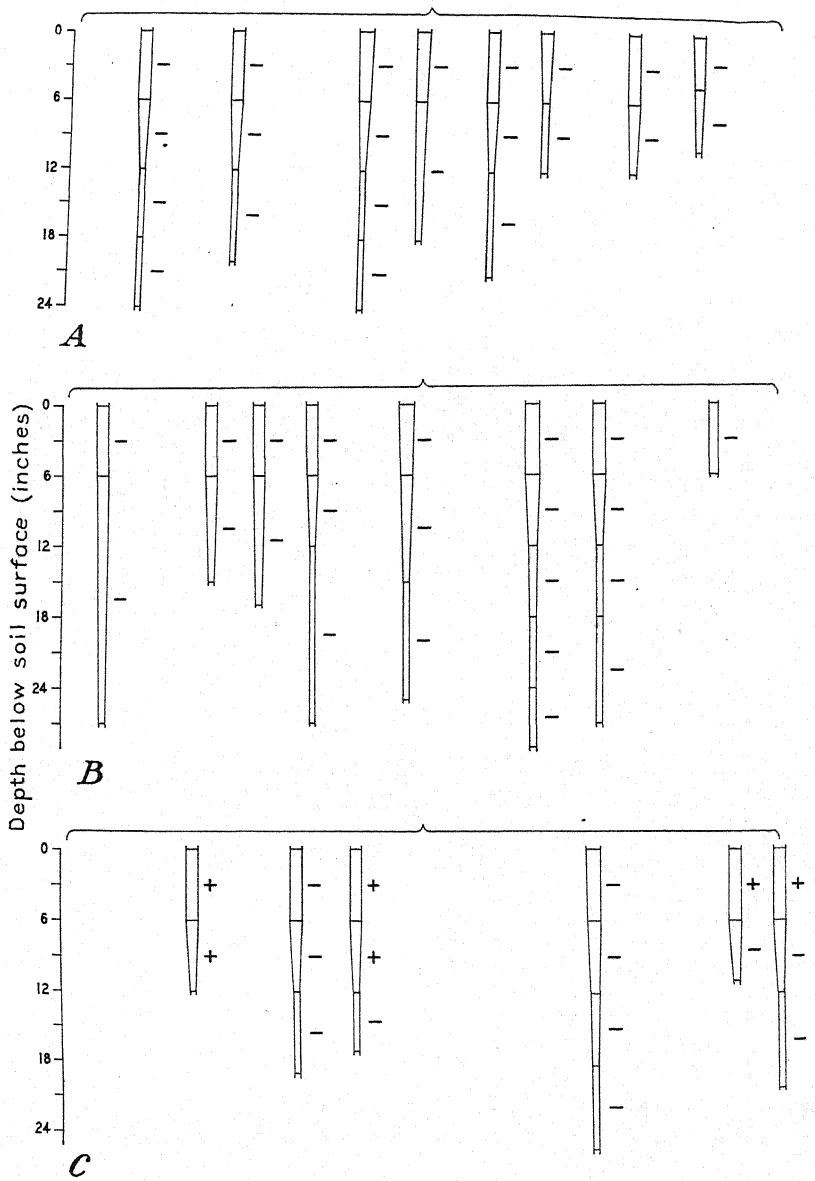


FIGURE 2.—Diagrammatic summary of results in plots excavated 34 to 39 days after industrial xylol was applied in areas designated by braces. Cotton plants were located within the plots at approximate positions shown. Roots were cut into portions as indicated, later growth of *Phymatotrichum* strands from these portions in soil cultures being indicated by + and lack of growth by −: *A*, application at rate of 166 g per square foot; *B*, application at rate of 83 g per square foot; *C*, application at rate of 41.5 g per square foot.

that it was still alive on portions of roots from the untreated check, and on roots from plants immediately adjoining the treated area. Growth of *Phymatotrichum* resulted also from portions of roots from adjoining rows, only 1½ feet distant from the side of the treated areas. Similar additional tests with roots of plants from outside the treated areas were made on each of the three dates of harvest, and it was found uniformly that very little if any toxic effect had occurred more than a few inches horizontally beyond the areas in which the tetrachlorethane had been applied.

Downward permeation of the tetrachlorethane through the soil was evidenced by direct observation of the odor while the roots were being dug, as well as by the indirect evidence of the effect on the fungus. In at least one instance, the tetrachlorethane odor was strong in soil at a depth of 28 inches, after 3 weeks. The odor was still noticeable in the soil at the end of the experiment, after 32 days.

In this experiment, tetrachlorethane applied at 166 g per square foot, in holes only 6 inches deep, was successful in killing or at least permanently inhibiting later growth of the fungus on or within affected cotton roots down to a depth of at least 2 feet. Application in four holes per square foot was evidently sufficient for reasonably uniform permeation despite the dense subsoil.

APPLICATION OF TETRACHLORETHANE AND XYLOL AROUND COTTON PLANTS, IN 1934

A more extensive series of tests was run in the fall of 1934 in the same cotton field at College Station. On August 29 and 30, 24 comparable areas were marked off on the advancing edges of root rot spots in this field. As before, each plot was carefully located to include some plants already showing signs of root rot and others as yet without above-ground symptoms. Each plot extended 5 feet along the row and 2 feet to each side. Applications were made on 18 plots, the remaining 6 serving as checks. Duplicate plots were used, the applications being at the rate of 166, 83, and 41.5 g per square foot for industrial xylol, commercial refined tetrachlorethane, and crude tetrachlorethane. Roots from half the plots were excavated after 15 to 19 days, and from the other plots after 34 to 40 days. These plots were excavated usually to depths of 3 feet or more, but few roots were recovered at greater depths than 24 to 28 inches (fig. 2).

The results of this experiment are summarized in table 2. At 166 g per square foot perfect control was obtained on deep and shallow portions of the roots with all three fungicides. At 83 g per square foot, the results were similar except that *Phymatotrichum* growth was obtained from 1 of the 112 portions of roots tested. At the lowest concentration, 41.5 g per square foot, the fungus remained alive in one root exposed to crude tetrachlorethane and in several roots exposed to xylol (fig. 2), and grew from 7 of the 79 portions tested. In the accompanying tests of untreated check plants, the fungus grew from 40 of the 111 root portions tested.

The roots excavated after 39 days from the plot treated with xylol at 41.5 g per square foot yielded a higher percentage of living *Phymatotrichum* than did roots from untreated plots excavated after the same interval. This is possibly to be explained as follows: In check plots, the viability of the fungus decreased in general with time (tables 1

and 2) as a result of gradual decay of roots and subsequent death of the fungus. But in the plot mentioned, continued decay of roots presumably was held in check by the xylol, which, however, was not maintained at a concentration sufficient to kill the fungus. With gradual dissipation of the xylol during the 39-day interval, the fungus was therefore still viable. Similar apparently favorable effects of low concentrations of fungicides have sometimes been found in laboratory tests with organic mercuries, which at concentrations too low to impede the growth of *Phymatotrichum omnivorum* frequently encourage more extensive growth than in the accompanying untreated checks.

TABLE 2.—*Effects of applying refined and crude tetrachlorethane and industrial xylol at different rates around cotton plants with root rot, August 1934*

Fungicide	Rate of application per square foot	Interval before excavation of roots	Plants in plot	Results of testing portions of roots in jars of moist soil		
				Portions tested	Portions yielding *Phymatotrichum omnivorum*	
	Grams	*Days*	*Number*	*Number*	*Number*	*Percent*
Tetrachlorethane	166	15	8	16	0	0
		34	9	26	0	0
	83	16	11	23	0	0
		35	8	17	0	0
	41.5	19	5	10	0	0
		39	7	19	0	0
Tetrachlorethane, crude	166	16	8	16	0	0
		34	8	21	0	0
	83	16	6	10	0	0
		36	9	28	1	4
	41.5	19	5	7	1	14
		40	10	15	0	0
Xylol, industrial	166	15	7	15	0	0
		34	8	22	0	0
	83	16	7	12	0	0
		34	8	22	0	0
	41.5	19	6	11	0	0
		39	6	17	6	35
None (checks)		16	5	10	2	20
		17	8	22	19	86
		19	9	16	12	75
		34	9	27	4	15
		39	9	21	2	10
		39	8	15	1	7

In general, these results agreed with those obtained in laboratory studies with materials applied on the surface of soil in open jars (*1, table 8*). Under these conditions, a concentration of 500 parts per million proved too low for inhibition of growth of the fungus, while higher concentrations were effective. Similarly, under the conditions of the present experiment, applications of between 83 and 166 g per square foot of any of the fungicides used will probably be necessary. The present experiment confirmed the test of the previous year in indicating that treatments of this kind could kill the root rot fungus in and on cotton roots well below the depth at which the fungicides were applied.

APPLICATION OF PENTACHLORETHANE AROUND A CHINABERRY TREE, IN JULY 1934

A 10-year-old chinaberry (*Melia azedarach* L.) tree, just beginning to wilt from an attack of root rot, was used for the following experi-

ment. An area 8 by 16 feet at one side of the tree was treated with pentachlorethane at the rate of 166 g per square foot. The soil at the other side of the tree was left untreated. Plots approximately 5 by 5 feet were excavated after 3 and 6 weeks from the treated and from the check areas. The roots within the plots were removed to a depth of about 5 feet, sawed into convenient portions, and placed as usual in soil cultures.

A condensed summary of the results is given in table 3. Of the roots from the treated area, only one portion one-half inch in diameter, taken after 3 weeks from a depth of 33 to 39 inches, yielded viable *Phymatotrichum*. Other infected roots of various diameters up to 2 inches and located to a depth of 5 feet in the treated area failed to yield growth of the fungus. This is of less significance, however, since the deeper roots from the untreated, check area also failed to yield growth, the growth recorded coming from roots at a depth of 12 to 18 inches.

APPLICATION OF PENTACHLORETHANE AROUND AN ELM TREE IN AUGUST 1934

A 9-year-old elm (*Ulmus pumila* L.) tree just showing signs of root rot was used in this experiment. An area 8 by 16 feet at one side of the tree was treated with pentachlorethane at the rate of 83 g per square foot. Roots were excavated after 31 days from a 6- by 6-foot plot within this treated area and from a similar plot on the other side of the tree. The results are given in table 3.

TABLE 3.—*Results of applying pentachlorethane at the rate of 166 g per square foot around a chinaberry tree and of 83 g per square foot around an elm tree, both of which were affected with root rot.*

Tree and location of plots	Interval before excavation of roots	Results of testing of portions of roots in jars of moist soil		
		Portions tested	Portions yielding *Phymatotrichum omnivorum*	
	Days	*Number*	*Number*	*Percent*
Chinaberry:				
Treated area	21	54	1	2
	42	33	0	0
Untreated area (check)	23	41	2	5
	43	33	6	18
Elm:				
Treated area	31	36	0	0
Untreated area (check)	31	42	22	52

The roots from the treated area, within which the fungus apparently was killed by the relatively light treatment, varied from ¼ inch to 2 inches in diameter, and were found at depths of 4 to 20 inches only, although the excavation was continued to about 5 feet. Strand growth from the untreated roots was from roots ⅛ to 3 inches in diameter, found at depths of 4 to 12 inches only. *Phymatotrichum* on the shallow roots of this elm tree was killed by application of pentachlorethane at 83 g per square foot.

ATTEMPTED ERADICATION OF ROOT ROT WITH TETRACHLORETHANE AND XYLOL

While the experiments summarized above indicated that the fungicides applied at 83 or 166 g per square foot would kill the root rot fungus on roots within the treated areas, they did not indicate whether such treatment would actually eradicate the fungus from infested areas. *Phymatotrichum* may survive on deeper roots than those recovered for testing, and it may survive also as sclerotia at various depths in the soil. The following experiment was therefore planned as a rather crucial test of the possibility of eradicating root rot by applying the fungicides by the method used in the other tests—that is, in holes punched to a depth of 6 inches.

The applications were made in plots bordered by "sorghum barriers." This precaution had not previously been used in experiments

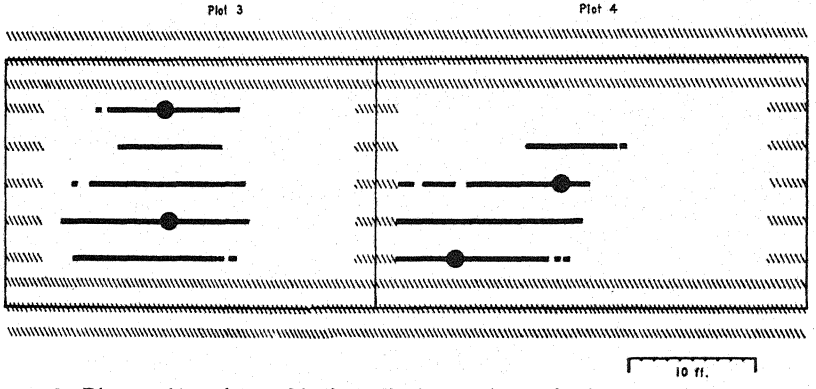


FIGURE 3.—Diagram of two plots used in the eradication experiment, showing extent of root rot in 1935. Continuous lines show edges of areas treated in 1934 with xylol and tetrachlorethane, respectively, and hachured lines show location of sorghum barrier rows. Dots indicate centers where root rot first recurred on July 26, 1935, and heavy lines the extent of root rot spread from these points by October 29, 1935.

on the eradication or control of *Phymatotrichum* root rot, and the value of such experiments had been weakened by the possibility that areas from which the fungus had been nearly or quite eradicated might periodically be reinfested from adjoining untreated areas. When cotton plants are used, this source of error can now be eliminated since it has been shown (7) that a few intervening rows of sorghum plants will act as a barrier to prevent spread of root rot from infected cotton plants into an immediately adjoining uninfested plot, over a period of years. A plot completely enclosed by rows of sorghum plants should, therefore, be safe from the spread of root rot from neighboring plots. This being so, it is possible to determine accurately the immediate or cumulative effect of attempted eradication or control methods. In the present experiment, the sorghum barriers along the sides of the plots consisted of three rows 2 feet apart. The ends of the plots were protected by planting sorghum for 3 feet at each end of each row of cotton. Each plot was thus enclosed completely by the sorghum border without interfering with cultivation (fig. 3).

TABLE 4.—*Synopsis of root rot eradication experiment by the use of xylol and tetrachlorethane in field plots of cotton guarded by sorghum barriers,*[1] *1934*

Plot No.	Fungicide, applied at 249 g per square foot	Time and conditions of application	Centers of infection appearing after the treatment	Results in 1935		
				Plants	Plants with root rot on—	
					Aug. 20	Oct. 29
			Number	*Number*	*Percent*	*Percent*
1	Xylol	Apr. 18–23, in wet soil	1	281	1.8	28.1
2	Tetrachlorethane	Apr. 2–9, in wet soil	3	336	0	13.4
3	Xylol	Aug. 9–11, in dry soil	2	295	4.4	47.5
4	Tetrachlorethane	Aug. 7–8, in dry soil	2	300	4.0	37.0
5	None (check)		(2)	523	99.8	100.0

[1] Plots 1 and 3 were each 20 by 30 feet in size; plots 2 and 4, 20 by 34 feet; and plot 5, 20 by 64 feet.
[2] About 55.

The experiment was located on plots that had served as check areas in a previous experiment. Root rot was introduced here by inoculation in 1929, and had become increasingly prevalent on cotton grown year after year, attacking nearly 100 percent of the plants in 1931, 1932, and 1933. The general plan of the present experiment included treatments with xylol and tetrachlorethane at the high rate of 249 g per square foot in wet soil in spring and in dry soil in summer (table 4). The applications in plots 1 and 2 were made before the crop was planted, in soil so wet that the fungicides were absorbed quite slowly from the holes into which they were poured. The applications in August were made after a long drought, and the fungicides were absorbed rapidly into the soil.

INJURY FROM THE FUNGICIDES

Cotton and sorghum were planted in these plots a month after the tetrachlorethane was applied in plot 2, and only 2 weeks after xylol was applied in plot 1. A good stand, with relatively little retardation of growth, was obtained in plot 2, but after repeated planting only occasional stunted plants were obtained in plot 1. In plots 3 and 4 the fungicides were applied around the growing cotton plants and caused severe injury. Within 1 month the plants in plot 4 were dead, and a month later all the plants in plot 3 had succumbed.

On March 7, 1935, small chinaberry trees were planted in each treated plot, and these as well as the cotton, planted April 1, showed residual injurious effects of the treatments. By June 29 the condition of the plants was as follows:

Plot 1, cotton plants 60 to 110 cm tall, chinaberry trees all alive.
Plot 2, cotton plants 15 to 25 cm tall, chinaberry trees all dead.
Plot 3, cotton plants 80 to 110 cm tall, chinaberry trees all alive.
Plot 4, cotton plants 60 to 95 cm tall, chinaberry trees 9 of 12 alive.

A full year after the tetrachlorethane was applied in the wet soil in plot 2, there was still severe injury, while the same material applied only 8 months previously in dry soil caused less injury in plot 4. Neither of the xylol-treated plots showed definite injury from the fungicide in 1935. Xylol was thus much less injurious than tetrachlorethane both in immediate and residual effects.

RECURRENCE OF ROOT ROT

Following the fungicide applications in 1934, root rot was observed only in plot 1. It appeared in October in a single center, and by December 10 had involved 6 of the 343 plants in the plot.

In 1935, the treated plots were still free of above-ground evidence of root rot until the end of June. By that time more than 20 percent of the plants in the check plot had been killed by the disease, which appeared at numerous points along each row. Root rot was observed in the treated plots on the following dates: Plot 1, July 6; plot 2, September 19; plot 3, July 26; and plot 4, July 26. It spread rapidly from only a few centers in each plot, and had involved a total of 30 plants by August 20, when all but one of the plants in the check plot had succumbed to the disease. However, the need of complete eradication to make fungicide treatment of value was well illustrated by the continued spread of root rot after this date over a large portion of the treated plots (table 4). Of 29 chinaberry trees that survived injury from the fungicides, 10 were killed by root rot in 1935.

The incidence of root rot in these treated plots was delayed and the prevalence of the disease reduced during the season following treatment, but the treatment failed to eradicate root rot. Possible causes of this failure are discussed below.

EFFECTS OF THE FUNGICIDES ON HOST PLANTS

Most of the experiments to determine the fungicidal value of the materials furnished little evidence as to their effect on the host plants, since the plants were dug out generally within a few weeks after treatment. Data were therefore obtained on this point by special treatments around some trees and shrubs growing in an experimental nursery at College Station, in small plots extending 10 feet along the rows and 2 feet to each side. During the summer of 1934, fungicides were applied within 20 such areas at 166, 83, and 41.5 g per square foot.

Of the three fungicides, tetrachlorethane was rapidly and severely injurious, xylol next, and pentachlorethane was least injurious. Crude tetrachlorethane was approximately as injurious as the refined tetrachlorethane, in a few tests. Rows of pomegranate (*Punica granatum* L.) bushes were treated at 166 g per square foot early in May. The tops of about 90 percent of those treated with pentachlorethane were apparently uninjured 6 months later, whereas most of those treated with tetrachlorethane were dead. Hackberry (*Celtis laevigata* Willd.) trees treated in May with tetrachlorethane and xylol at 166 g succumbed during July and August, respectively, while trees treated at the same rate with pentachlorethane were still apparently uninjured a year later. Similar results were obtained with some other plants.

The final effects of the various treatments are summarized in table 5. It will be noted that hackberry and retama (*Parkinsonia aculeata* L.) trees, and most of the pomegranate bushes, survived the highest rate of treatment with pentachlorethane without definite injury. At the same rate, the tetrachlorethane and xylol treatments killed hackberry trees and more than half of the pomegranate bushes, only the

retama proving resistant. In descending order of susceptibility to fungicide injury, the plants ranked approximately as follows:

Live oak (*Quercus virginiana* Miller), elm, ilex, hawthorn (*Crataegus*), hackberry, pomegranate, and retama.

TABLE 5.—*Summary of injurious effects produced within 1 year by applications of volatile fungicides in soil around woody plants in nursery rows*

Materials and rates applied (grams per square foot)	Plants killed	Plants surviving
Tetrachlorethane:		
166	Hackberry; ilex; pomegranate, 75 percent.	Retama; pomegranate, 25 percent.
83	Live oak; pomegranate, 70 percent.	Hackberry; pomegranate, 30 percent.
41.5		Hackberry; pomegranate.
Xylol:		
166	Hackberry; pomegranate, 55 percent.	Retama; pomegranate, 45 percent.
83	Elm; pomegranate, 20 percent	Hackberry; pomegranate, 80 percent.
41.5		Hackberry, hawthorn, pomegranate.
Pentachlorethane:		
166	Pomegranate, 30 percent	Hackberry; retama; pomegranate, 70 percent.
83	Pomegranate, 25 percent	Hackberry; pomegranate, 75 percent.
41.5		Hackberry.

A special comparison was made of xylol applied throughout an area 5 by 5 feet square around a hackberry tree, and in an area similar except that a 2- by 2-foot space was left untreated immediately around the trunk of the tree. The first tree showed yellowed leaves soon after treatment and was dead by the end of the season, whereas the second tree was apparently uninjured a year later.

In general, the three fungicides were injurious to some plants at the two higher concentrations, only the lower rate of 41.5 g per square foot proving generally safe. Since by the method of application used this lower rate was not uniformly effective against the root rot fungus on diseased plants, curative treatment of infected woody plants with these fungicides will probably be impractical. For further experiments, pentachlorethane would be suggested rather than either of the other materials, since of the fungicides tested it was least injurious to the plants and most effective against the fungus. In experiments aimed at eradication of root rot without particular consideration of plants already growing in the infested area, the less expensive [4] (although more injurious) tetrachlorethane or xylol might be given first choice. Tetrachlorethane may be expected to have a much longer residual injurious effect on future plantings than xylol, so xylol might somewhat arbitrarily be suggested as possibly the most promising of the three materials for further consideration in eradication experiments.

DISCUSSION

A chemical eradication method for use against *Phymatotrichum* root rot would be valuable at the present time for experimental and limited practical use only. It would be used, as was pointed out

[4] Pentachlorethane was available at about 15 cents, tetrachlorethane at 8 cents, and industrial xylol at about 4 cents per pound respectively.

earlier (*2*, *5*), chiefly for small root rot spots occurring in otherwise uninfested areas and for emergency eradication should the disease be carried to new areas. Such a method might later be of value also for eliminating final points of infestation in fields where partial eradication had been attained by other and less costly methods. In any case, practical use of fungicides for treatment of the soil would seem to be limited to materials and methods that can effect actual eradication of the fungus, since partial control would scarcely justify the cost of materials and labor. Complete eradication would be particularly desirable when planting susceptible trees and shrubs in previously infested soil, since even scattered infection by *Phymatotrichum* causes rapid loss of orchard and other susceptible perennial plantings (*8*).

The experiments reported above included field tests of pentachlorethane, tetrachlorethane, and xylol, applied always in holes 6 inches deep and covered only by pushing soil into the holes. Treatments around infected plants in small plots, as summarized in tables 1 to 3, indicated that under these conditions applications of 83 to 166 g per square foot regularly killed the fungus on and within roots of the plants down to the depth recovered. Of 253 portions of roots tested from plots treated at the higher rate, the fungus was viable only on 1 portion taken from a depth of 33 to 39 inches. With this exception, infected roots recovered to depths of 2 feet or more did not yield growth of *Phymatotrichum* strands, although the fungicides had been applied in holes only 6 inches deep. These field results thus confirmed the preliminary laboratory results which had suggested that the materials used here were capable of rapidly permeating the soil as well as killing the root rot fungus in approximately the concentrations provided by these applications.

The high cost of chemical treatment of the soil would be justified for therapeutic treatment of valuable diseased trees and shrubs if the fungicides could be used without injury to the host plants in concentrations sufficient to kill the fungus. The applications reported in the present paper suggest that this will probably not be possible, at least by the method of application used in these experiments. In general, applications at 83 or 166 g per square foot injured some of the plants tested. The lower rate, 41.5 g per square foot, did not injure the plants used in these tests, but it failed to kill the fungus in some cases. Curative treatment of infected woody plants with these fungicides will probably be impractical.

The possibility of using tetrachlorethane or xylol for eradicating root rot from infested plots was explored in a separate experiment in which the freedom from root rot of the succeeding crops was used as the criterion rather than the death of the fungus on roots recovered from treated plots. Root rot was markedly diminished in the treated plots, but recurred in each case from a few scattered points. Since the experimental plots were adequately guarded by sorghum barriers, this recurrence of root rot well inside the treated plots was due not to re-encroachment of the fungus from surrounding untreated areas, but to failure of the treatment to eradicate the fungus from the plots. This might be explained in several ways: (1) It might be assumed that the fungus survived as sclerotia or on roots located at a greater depth than that reached effectively by fungicides applied

in holes 6 inches deep; or (2) it might be that even at the depths reached by the fungicides they were not able to kill the fungus under all the soil conditions encountered; or (3) continuing evaporation of the fungicides from the surface soil might have prevented an effective concentration from being maintained, particularly at points fairly near the surface. Results in line with this third hypothesis were obtained in laboratory experiments with open jars exposed to a current of air (*1, experiment C20*). Similarly, Godfrey and his associates (*3, 4*) found in experiments for the control of nematodes, that to prevent rapid evaporation of chloropicrin from the soil it was necessary to confine it by a cover of some impervious material. Paper coated with glue was more satisfactory than other soil coverings tested.

It seems possible, then, that failure of tetrachlorethane and xylol to eradicate root rot in the treated plots may have been due more to evaporation of the fungicides from the surface than to failure of these materials to be sufficiently fungicidal or to penetrate the soil adequately. Further experiments might show that covering the surface of the soil would so increase the efficiency of these materials that they would be able to eradicate the root rot. Meanwhile, despite the promising results obtained from applications of these fungicides around infected plants, the other results recorded do not justify recommending them for practical use against *Phymatotrichum* root rot.

SUMMARY

In field tests against root rot caused by *Phymatotrichum omnivorum*, pentachlorethane, tetrachlorethane, and xylol were applied in holes pierced to a depth of 6 inches in the soil, and covered only by pushing soil into the holes.

These materials were applied first in small plots around infected cotton plants and chinaberry and elm trees. After various periods, the efficacy of the materials in killing the fungus on roots was determined by excavating the roots to a depth of 2 feet or more and culturing portions in jars of soil. Roots recovered from plots treated at 166 g per square foot yielded *Phymatotrichum* growth from only 1 of 253 portions; plots treated at 83 g per square foot from 2 of 210 portions; plots treated at 41.5 g per square foot from 7 of 79 portions; and untreated check plots from 92 of 293 portions. The fungicides were effective to at least 2 feet below the depth at which they were applied.

Xylol and tetrachlorethane were applied at 249 g per square foot in an experiment designed to test the ability of these materials to eradicate root rot from infested plots. The plots were bordered by "sorghum barriers" to prevent re-encroachment of root rot from adjoining untreated areas. Incidence of root rot was markedly delayed and its prevalence reduced during the season following treatment, but the disease recurred in each plot from isolated centers of infection.

Applications around growing trees and shrubs showed that tetrachlorethane was rapidly and severely injurious to host plants, xylol next, and that pentachlorethane was least injurious. In general, applications at 166 or 83 g per square foot injured some of the plants, but no injury was found after treatment at 41.5 g per square foot.

The order of susceptibility to fungicide injury, of the plants tested, was approximately as follows, the more susceptible being listed first: Live oak, elm, ilex, hawthorn, hackberry, pomegranate, and retama. Residual injurious effects on crops planted later were much more severe after treatment of soil with tetrachlorethane than after treatment with xylol.

Failure of tetrachlorethane and xylol to eradicate root rot completely from infested plots was possibly due more to loss of the fungicides from the surface soil than to failure of these materials to be sufficiently fungicidal or to penetrate the soil adequately. Further experiments with the surface of the soil covered to lower evaporation losses are suggested. Meanwhile, the results do not justify recommendation of these soil fungicides for practical use against *Phymatotrichum* root rot.

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A CYTOLOGICAL STUDY OF HOST-PARASITE RELATIONS OF VENTURIA INAEQUALIS ON APPLE LEAVES[1]

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INTRODUCTION

Venturia inaequalis (Cke.) Wint. is representative of a group of phytopathogenic fungi characterized by a very distinctive type of parasitism. Though its invading mycelium is characteristically limited to a subcuticular position, nevertheless the fungus has intimate relations with the underlying host tissues, from which it derives substantial nutriment and upon which it exerts important influences. The end results of infection in terms of the efficiency of fungous nutrition and the range of congeniality of host-parasite relations are very similar to those of invasion by many of the so-called obligate parasites.

Though excellent cytological studies have been made on the host-parasite relations of various phytopathogenic fungi, especially the Uredinales (e. g., *3, 4, 24, 27, 28, 29*),[2] comparatively little attention has hitherto been given to similar studies within the group of which *Venturia inaequalis* is typical. The classical contributions of Aderhold (*1, 2*) laid the foundation for such investigations. Wiltshire's (*33*) excellent study substantially supplemented Aderhold's work, especially with reference to the details of penetration of the cuticle and the early phases of establishment of the parasite in the subcuticular position. Little detailed consideration, however, has been given to the distinctive phenomena of parasitism and pathogenesis in the later stages of the host-parasite relations.

The investigation reported herein (*15*) was undertaken in the hope of contributing to the description and interpretation of the phenomena of parasitism of *Venturia inaequalis* and the resistance offered by certain of its hosts. This work is an outgrowth of a series of studies of apple scab and related problems that has been in progress at Wisconsin for some years (*7, 8, 11, 12, 14, 16, 17, 19, 20, 21, 32*), and is a companion study to an investigation on apple rust (*18*), in which is given a brief review of literature pertinent to both lines of work. Discussions of literature relating especially to the initial stages of infection by *V. inaequalis* are given by Wiltshire (*33*) and Keitt and Jones (*14*). Brown and Harvey (*6*), Brown (*5*), and Rice (*23*) have reviewed the literature of the broader field of host-parasite relations.

MATERIALS AND METHODS

Two monoconidial isolates of *Venturia inaequalis* were employed. These cultures were originally isolated by Palmiter (*20*), and his designations are retained in this paper. Isolate 17 was obtained from

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[2] Reference is made by number (italic) to Literature Cited, p. 617.

infected Fameuse apples (*Malus sylvestris* Mill.) and 22 from infected Delicious apples, both from Wisconsin orchards. These isolates were chosen because they sporulated well on artificial culture media and differed in their capabilities of infecting the foliage of certain apple varieties.

Having been carried in culture for several years without host passage, each isolate was inoculated on apple leaves, and monoconidial reisolations were made by Keitt's (*10*) method. The reisolates conformed closely to Palmiter's descriptions, except that they sporulated more abundantly in culture and seemed to show certain differences from his results in degree of infection. The latter are attributable in part to differences in the methods of classification employed. However, the possibility that the isolates may have undergone some change in culture is recognized.

The apple varieties employed were Fameuse, Yellow Transparent, and Missouri Pippin. These had been included in Palmiter's infection studies and found to react differently when inoculated with the two isolates mentioned above. Preliminary infection experiments with the reisolates showed that these varieties were suitable for the present investigation.

Two-year-old nursery apple trees were placed in galvanized-iron containers in about 8 kg of soil (3 parts compost soil and 1 part sand), adjusted, and held at 70 percent of the maximal water-holding capacity (*31*, v. 1, p. 150). After being rooted in a cool basement for 2 to 3 weeks, they were placed on a greenhouse bench. All trees were kept in the same greenhouse under comparable environmental conditions. Two shoots were allowed to develop on each tree. When the shoots were about 15 to 20 inches long and showed 14 to 18 leaves, they were inoculated. At the time of inoculation, a bit of string was tied about the petiole of the youngest leaf of each shoot to facilitate identification of the inoculated leaves after further growth had occurred. Fumigation was employed as necessary to control insects.

The spores used for inoculation were produced by cultures grown on cheesecloth "wicks" saturated with nutrient solution in 12-ounce medicine bottles. A 15- by 8-cm strip of cheesecloth was placed in each bottle with about 10 cc of nutrient solution (3 percent Trommer's malt extract), so that it formed a closely adherent lining for one of the walls. The bottles were then sterilized in an autoclave. About 10 days before the spores were needed, the sterile nutrient solution in the bottles was seeded with a few drops of a suspension of conidia, introduced by means of a sterile pipette. The bottles were laid in a horizontal position for about 30 minutes to allow the spores to settle on the cheesecloth, then placed upright in an incubator at 20° C. for 6 days, and after that transferred to a 16° incubator where they remained for from 4 to 6 days. The lower temperature favored sporulation.

When a spore suspension was needed for inoculation, the pieces of cheesecloth were removed from the bottles and the spores washed off by atomizing with water. The suspension thus obtained was centrifuged, the supernatant liquid decanted, and water added. In this way a suspension of washed spores relatively free from fungal staling products or nutrient materials could be obtained in any desired concentration. Sterile distilled water and sterile glassware and atomizers were used.

During the greenhouse season six series of inoculations were made, the first on March 23 and the last on May 6. Series 1, 2, 3, 4, and 6 were made with conidia from culture. Series 5 was made with spores washed from sporulating scab lesions on apple leaves infected with the designated isolates for comparison. In each case the inoculations with the different isolates were made separately and suitable precautions were taken to insure against contamination.

Each inoculation was performed in duplicate or quadruplicate, depending on the material available. With the exception of series 2 and 4, four trees of each variety were inoculated with each isolate. In each lot 2 trees were heavily inoculated (a drop of spore suspension when placed on a glass slide under the microscope showing approximately 150 spores per low-power field) and the remaining 2 were lightly inoculated (10 spores per low-power field). The heavily inoculated leaves were intended for study of the early stages of infection, whereas the light inoculation was planned for studying the older lesions. One of the lightly inoculated trees of each isolate-variety combination was reserved for study of macroscopic development of the disease.

Prior to the application of inoculum each tree was thoroughly washed with a fine spray of tap water from an angled spray nozzle, gently shaken to remove the larger drops, and allowed to dry.

The spore suspensions were applied to the upper, or ventral, surface of all the leaves on each shoot by means of a De Vilbiss atomizer which had previously been throughly cleaned and steamed for 15 minutes. Only one tree was inoculated at a time in a room remote from the greenhouse in which the remaining trees were kept. After inoculation the trees were placed for 36 hours in a moist chamber described by Keitt et al. (*13*, fig. 20) at 16° C. They were then returned to the greenhouse.

Fixations were made at regular intervals after inoculation. Material was collected daily for the first 6 days and at longer intervals for the next 4 weeks. Two earlier collections were made at 14- and 20-hour intervals, respectively, after inoculation in order to obtain the early stages of penetration. To promote uniformity of results daily fixations were made at 4 p. m. and material was always taken from the third or fourth leaf below the one marked with string.

In a preliminary experiment, various fixatives were tried in combinations with different methods of dehydrating and staining. A formal-chrom-acetic mixture (1-percent aqueous solution of chromic acid, 100 cc; glacial acetic acid, 4 cc; 40-percent aqueous solution of formaldehyde, 50 cc; freshly prepared for each fixation) suggested in correspondence by G. H. Conant gave the best results and was used throughout. The n-butyl alcohol method of Zirkle (*34*) was employed for dehydration and embedding. Sections were cut 6μ thick. Heidenhain's iron-alum haematoxylin was a satisfactory stain for the early stages of penetration because it brought out the infection hypha clearly. A modification of Flemming's triple stain consisting of safranine, gentian violet, and fast green was best for the study of host-parasite reactions. In studying the relationships of the fungus to the cuticle and the walls of the epidermal cells, sections stained in Delafield's haematoxylin and Sudan IV and mounted in glycerin were useful.

A modification of the cleared-leaf method of Peace (*22*) was well adapted to a survey of certain aspects of conidial germination, direct cuticular penetration, and the establishment of the fungus beneath the cuticle, as it afforded a means of observing these phenomena in toto on relatively large areas of leaf surface. Bits of leaf were collected 14, 24, 48, 96, and 144 hours, respectively, after inoculation, fixed in acetic alcohol, cleared in chloral hydrate, and stained with acid fuchsin in lactophenol.

After each inoculation, material was collected for fixation and clearing as described above. The data reported in this paper, however, are taken chiefly from series 3 because of the very favorable circumstances that attended this experiment. The trees were growing uniformly and vigorously and were entirely free from greenhouse insects.

MACROSCOPIC OBSERVATIONS ON LIVING LEAVES

At suitable intervals after inoculation, data were taken on the appearance and development of infection on the individual leaves of one shoot from each of the trees that were set aside for this purpose. The results, shown in table 1, are also representative of the infection that developed on the trees from which material was taken for microscopic study.

Isolate 22 was highly pathogenic on Yellow Transparent, strikingly less virulent on Fameuse, and only slightly pathogenic on Missouri Pippin.

On the young leaves of Yellow Transparent, infection became macroscopic the ninth day following inoculation, and the incubation period on the older leaves was progressively longer (*14*). These spots, at first small, indistinct, and slightly olivaceous, increased rapidly in diameter and began to produce conidia abundantly on about the fourteenth day after inoculation. Necrosis began to appear in the middle of these lesions about 5 days after the first sporulation. The necrotic area spread rapidly for the next 10 days, after which nearly all of the visible lesion was dead. Necrosis always followed sporulation and neither phenomenon occurred on leaves below the sixth (the one marked with string being no. 1). Development apparently ceased in all the lesions in from 30 to 36 days after inoculation.

On Fameuse, infection first appeared about 12 days after inoculation, and was confined to the younger leaves near the tip of the shoot. The lesions remained small (less than 5 mm in diameter), produced no conidia, and necrosis was not apparent. About 3 weeks after inoculation, development apparently ceased, and the lesions began to change somewhat, becoming bronzed or reddish brown.

On the leaves of Missouri Pippin, no macroscopic signs of infection were detected until 18 days after inoculation, when minute, barely visible flecks appeared. These flecks seemed to be more or less grouped together in small patches a millimeter or less in diameter. These manifestations might easily have been confused with certain insect punctures. Because the plants studied were free from insects, however, it was thought, and later proved by the cytological study, that the flecks actually were scab lesions.

The symptoms that developed when isolate 17 was used with the same three varieties were distinctly different from those just described.

TABLE 1.—*Occurrence of macroscopic lesions on the ventral surface of apple leaves of series 3, inoculated with Venturia inaequalis, Apr. 17, 1935,*

Isolate and variety	Dates noted	Lesions on stated leaves, numbered serially from base to tip of shoot																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
', Yellow Transparent	Apr. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(1)	(2)	(2)	------
	Apr. 29	0	0	0	0	0	0	0	0	0	0	0	8	26	(2)	(2)	(2)	(2)	------
	May 1	0	0	0	0	0	0	0	0	4	17	14	26	(1)	(2)	(2)	(2)	(2)	------
	May 5	0	0	0	0	0	0	0	7	28	(1)	(1)	(1)	(1)	(2)	(2)	(2)	(2)	------
	May 10	0	0	0	0	0	8	0	7	(1)	(1)	(1)	(1)	(1)	(2)	(2)	(2)	(2)	------
	May 16	0	0	0	0	0	8	13	17	(1)	(1)	(1)	(1)	(1)	(2)	(2)	(2)	(2)	------
	May 25	0	0	0	0	0	8	13	17	(1)	(1)	(1)	(1)	(1)	(2)	(2)	(2)	(2)	------
, Yellow Transparent	Apr. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	------	------
	Apr. 29	0	0	0	0	0	0	0	0	0	0	0	0	0	8	(1)	------	------	------
	May 1	0	0	0	0	0	0	0	0	0	0	0	0	0	(1)	(2)	------	------	------
	May 5	0	0	0	0	0	0	0	0	0	0	7	33	4	(1)	(2)	------	------	------
	May 10	0	0	0	0	0	0	0	0	0	(1)	16	40	(1)	(1)	(2)	------	------	------
	May 16	0	0	0	0	0	0	0	0	0	(1)	(1)	(1)	(1)	(1)	(2)	------	------	------
	May 25	0	0	0	0	0	0	0	0	0	(1)	(1)	(1)	(1)	(1)	(2)	------	------	------
, Fameuse	Apr. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Apr. 29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)
	May 1	0	0	0	0	0	0	0	0	0	0	0	0	26	(1)	(1)	(2)	(2)	(2)
	May 5	0	0	0	0	0	0	0	0	0	0	15	16	(2)	(2)	(2)	(2)	(2)	(2)
	May 10	0	0	0	0	0	0	0	0	12	33	(1)	(1)	(2)	(2)	(2)	(2)	(2)	(2)
	May 16	0	0	0	0	0	0	0	0	(1)	(1)	(1)	(1)	(2)	(2)	(2)	(2)	(2)	(2)
	May 25	0	0	0	0	0	0	0	0	(1)	(1)	(1)	(1)	(2)	(2)	(2)	(2)	(2)	(2)
, Fameuse	Apr. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	------	------	------
	Apr. 29	0	0	0	0	0	0	0	0	0	0	0	26	(1)	(1)	------	------	------	------
	May 1	0	0	0	0	0	0	0	0	0	0	0	(1)	(2)	(2)	------	------	------	------
	May 5	0	0	0	0	0	0	0	0	0	0	10	(1)	(2)	(2)	------	------	------	------
	May 10	0	0	0	0	0	0	0	0	0	0	24	(1)	(2)	(2)	------	------	------	------
	May 16	0	0	0	0	0	0	0	0	0	0	24	(1)	(2)	(2)	------	------	------	------
	May 25	0	0	0	0	0	0	0	0	0	0	24	(1)	(2)	(2)	------	------	------	------

See footnotes at end of table.

TABLE 1.—*Occurrence of macroscopic lesions on the ventral surface of apple leaves of series 3, inoculated with Venturia inaequalis, Apr. 17, 1935*—Continued

Isolate and variety	Dates noted	Lesions on stated leaves, numbered serially from base to tip of shoot																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
17, Missouri Pippin	Apr. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	------
	Apr. 29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	------
	May 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	------	------
	May 5	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	(2)	(2)	------	------
	May 10	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	(2)	(2)	------	------
	May 16	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	(2)	(2)	------	------
	May 25	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	(2)	(2)	------	------
22, Missouri Pippin	Apr. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	------	------
	Apr. 29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	------	------
	May 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	------	------
	May 5	0	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	------	------	------
	May 10	0	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	------	------	------
	May 16	0	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	------	------	------
	May 25	0	0	0	0	0	0	0	0	0	0	0	0	0	(2)	(2)	------	------	------

[1] More than 50 lesions.
[2] Lesions numerous, but not clearly enough defined to count.

TABLE 2.—*Data pertaining to the inception of infection of apple leaves by Venturia inaequalis in the several isolate-variety combinations studied* [1]

Isolate and variety	Development after—																	
	14 hours			24 hours					48 hours					96 hours				
	Germinated	Appressoria	Pores	Germinated	Apressoria	Pores	Primary stromata	Average diameter of primary stromata	Primary stromata	Average diameter of primary stromata	Secondary hyphae	Average diameter of lesions	Epidermal cells necrotic	Primary stromata	Average diameter of primary stromata	Lesions	Average diameter of lesions	Epidermal cells necrotic
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Microns*	*Number*	*Microns*	*Number*	*Microns*	*Number*	*Number*	*Microns*	*Number*	*Microns*	*Number*
22, Yellow Transparent	38	0	0	47	55	51	22	18.5	66	55	362	176	0	59	75.3	46	356.6	0
17, Yellow Transparent	40	0	0	46	66	62	4	9.0	75	25.7	236	90.5	0	71	37.7	45	223.0	0
17, Fameuse	44	0	0	50	80	73	41	13.0	68	41.0	297	137.3	0	76	55.4	49	341.0	0
22, Fameuse	34	0	0	46	51	49	10	7.5	58	17.0	228	114.8	0	65	44.6	46	208.0	0
17, Missouri Pippin	45	6	0	49	77	60	32	10.0	54	23.3	178	92.3	0	59	36.4	46	193.0	0
22, Missouri Pippin	37	0	0	43	48	46	40	6.0	51	19.0	129	90.8	86	58	37.3	42	164	116

[1] In each case the data were from 50 conidia taken at random on the ventral surface of young cleared leaves studied in toto.

In the case of Yellow Transparent and Fameuse, the degree of severity of infection by isolate 17 was just the converse of that by 22, 17 being intermediately or mildly parasitic on the leaves of Yellow Transparent and highly parasitic on Fameuse. Infections by 17 on Fameuse and 22 on Yellow Transparent were closely similar with reference to incubation period, sporulation, and general development and appearance of the lesions. Infections by 17 on Yellow Transparent and 22 on Fameuse were also comparable in the degree of development. Missouri Pippin was decidedly resistant to isolate 17 as well as 22, although the manifestations of resistance were different in these two isolate-variety combinations. As mentioned above, isolate 22 induced minute flecks on Missouri Pippin leaves about 18 days after inoculation. In contrast, isolate 17 on the leaves of this variety induced macroscopic lesions in from 12 to 15 days after inoculation. These lesions were irregular and diffuse in outline and attained a diameter of 1 cm or more. They were pale green and easily discernible, in contrast to the dark-green color of the normal Missouri Pippin leaf. Apparently there was no sporulation or development of necrosis.

EARLY STAGES OF INFECTION

CLEARED-LEAF STUDY

The phenomena of the germination of conidia, formation of appressoria, and penetration of the cuticle, as observed on cleared leaves examined in toto, appeared to be similar in all the isolate-variety combinations. Following penetration, however, there were distinctive differences in the extent and nature of the development of the mycelium in the subcuticular position. Results from microscopic counts and measurements made on material of each isolate-variety combination collected at intervals of 14, 24, 48, and 96 hours, respectively, after inoculation are shown in table 2.

At the end of the 14-hour period, germination of most of the conidia had begun. The conidia of both isolates were usually two-celled. In this early stage of germination, the distal cell of the spore had expanded and developed a slight protuberance. The spore wall, however, remained intact, forming a thin, slightly stained membrane about the protuberance.

At the end of the 24-hour period, the process was decidedly farther advanced. The rudimentary germ tubes, usually from the distal cell as described above, had bulged at the tip to form characteristic appressoria, closely adherent to the cuticle. Occasionally a germ tube branched to form two distinct appressoria, each of which functioned in actual penetration of the host. Penetration from the proximal cell of the conidium was less common. It was observed to take place in some cases without the development of a germ tube or characteristically differentiated appressiorium (*14*), the cell itself being modified to function as an appressorium (fig. 1, *D*).

EXPLANATORY LEGEND FOR FIGURE 1

FIGURE 1.—Germination of conidia, formation of appressoria, and early development of infection from cleared leaves examined in toto: *A*, Isolate 22 on Yellow Transparent 24 hours after inoculation; spore (*s*), appressorium with penetration pore (*a*), and young amoeboid primary hypha (*ph*). × 900. *B*, Isolate 22 on Yellow Transparent 36 hours after inoculation; proliferation of cells in primary hypha (*ph*), young stolonlike hyphae (*st*). × 900. *C*, Isolate 22 on Yellow Transparent 4 days after inoculation; primary fungal stroma (*ps*), secondary lateral branches forming at *sb*, stolonlike hyphae (*st*), lateral branch initials (*b*), and secondary stromata (*ss*). × 400. *D*, Isolate 17 on Missouri Pippin 4 days after inoculation; primary stromata (*ps*) and stolonlike branches (*st*) less vigorous in appearance than those in *C*. × 400. *E*, Isolate 22 on Missouri Pippin 2 days after inoculation; epidermal cells showing necrosis are represented in solid black. × 400. *F*, Isolate 22 on Missouri Pippin 4 days after inoculation; epidermal cells showing necrosis are represented in solid black. × 400.

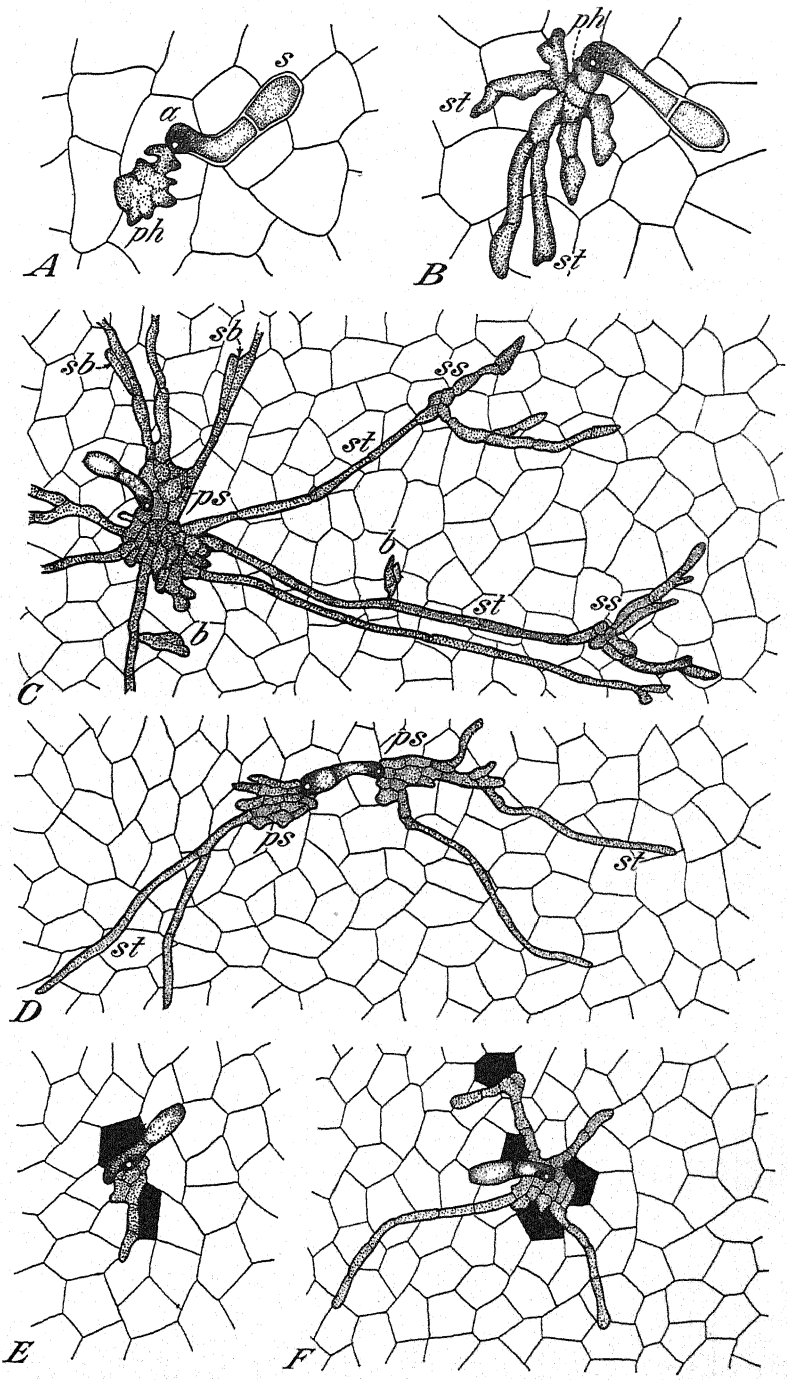


FIGURE 1.—Germination of conidia, formation of appressoria, and early development of infection from cleared leaves examined in toto. (For explanatory legend see opposite page.)

In the wall of the appressorium in contact with the leaf surface a characteristic transparent porelike area, usually about 2μ in diameter (p. 603), indicated the place of direct cuticular penetration. In some preparations very careful focusing with an oil-immersion objective seemed to show the presence of the infection hypha itself at or near the middle of the transparent area. This image, however, was not distinct enough to be interpreted with absolute certainty. The appearance of the primary hypha beneath the cuticle, however, was definite proof that penetration had occurred. At the end of the 24-hour period, the isolate-variety combinations of 22 on Yellow Transparent and Missouri Pippin and 17 on Fameuse and Missouri Pippin showed high percentages of completed penetration. In cleared-leaf preparations, the primary hypha was hyaline and only very slightly stained with the acid fuchsin. Unless it protruded beyond the overlying appressorium, it was masked by this structure and, consequently, overlooked. In view of this fact, the figures in table 1 recording the occurrence of primary hyphae represent the minimal rather than the maximal number of actual entries that had occurred at the time. A typical case showing a germinating conidium, appressorium, pore and primary hypha is shown in figure 1, *A*.

The single-celled, irregularly shaped primary hyphae, which were found in most 24-hour-old preparations, rapidly increased in size, due to cell proliferation and enlargement, and developed into primary fungal pads or stromata. Secondary hyphal branches radiated out from the periphery of the primary stromata. A 36-hour preparation (fig. 1, *B*) illustrates these phenomena.

Data taken at the end of the 48-hour period show that the development of each isolate on its most susceptible host variety had markedly exceeded that of the same isolate on less susceptible varieties. For example, with reference to the size of the primary stromata, the number of secondary hyphae, and the diameters of the lesions, isolate 22 on Yellow Transparent, closely followed by 17 on Fameuse, was much more advanced than 17 on Yellow Transparent or 22 on Fameuse. Thus far, each of the isolates had developed only very feebly on the resistant variety Missouri Pippin. With isolate 22 on this variety, epidermal cells underlying the spreading hyphae showed brown discoloration, a manifestation of necrosis that will be discussed in detail later. The position of the collapsed epidermal cells in relation to the feeble, somewhat gnarled hyphae is shown in the two camera lucida sketches in figure 1, *E* and *F*. Isolate 17, however, did not induce collapse of the underlying epidermal cells (fig. 1, *D*).

In contrast to their feeble mycelial growth on Missouri Pippin, both isolates developed in a typically dendritic fashion on Yellow Transparent (fig. 1, *C*) and Fameuse. Proliferation of the cells of the primary stroma (*ps*) continued and from its periphery long slender, stolonlike hyphae (*st*) radiated outward. These hyphae at first were single and unbranched, with widely separated septa. Lateral branches arose at very sharp angles as shown at *sb* or at right angles (*b*). The former type radiated out in the same fashion as the stolonlike secondary hyphae, while the latter type developed feebly to intensify the network or lattice arrangement of the fungal pattern. After the stolonlike hyphae had reached a distance of from 50μ to 100μ from the primary stroma, secondary stromata were produced. From these, tertiary branches radiated still farther. This process was repeated

several times during the spread of the lesion. In the isolate-variety combinations of 22 on Yellow Transparent and 17 on Fameuse, proliferation of the cells of these individual stromata often resulted in the development of a structure several cells thick, and it was from these stromata that conidiophores penetrated the cuticle and produced spores in large numbers. In the combinations of 22 on Fameuse and 17 on Yellow Transparent, many conidiophores were formed but no conidia were observed.

At the end of the 96-hour period the most significant differences were apparent in the average diameter of the lesions. The differences that have been pointed out with reference to the material collected at the end of the 48-hour period were increased. In addition, the hyphae were robust in all lesions except on Missouri Pippin, on which they were slender and feeble.

PHENOMENA OF PENETRATION

The germinating conidia of *Venturia inaequalis* penetrated the cuticle of the apple leaf directly. The process was similar in all details for all isolate-variety combinations. There was no cytological evidence to show that the cuticular barrier of young leaves in any way hindered the establishment of the fungus, even in the least compatible isolate-variety combinations studied.

Actual cuticular penetration as well as the sequence of events preceding it were best observed and interpreted in preparations of material collected 20 hours after inoculation. For convenience several processes involved in penetration will be described separately as follows:

Germination of Condium

As described under the "cleared-leaf study" the germ tubes were very short or lacking and were usually observed as mere protuberances from the distal, or pointed, end of the spores. During the germination process, each cell of the spore contained one nucleus (fig. 2, *G*). The distal, or germinating, cell became densely filled with protoplasm, whereas the proximal cell appeared to be partly evacuated (fig. 2, *G, I, K, L*).

Formation of Appressorium

The advancing tip of the germ tube soon became closely adherent to the leaf surface and developed into a more or less clearly differentiated appressorium. Viewed in cross section, the appressoria appeared to be oval or rounded in outline and densely filled with cytoplasm (fig. 2, *A, B*). The protoplasm stained heavily, and in some cases the nuclei could not be identified. Longitudinal sections (fig. 2, *C, E, F, G, K*) clearly show that the appressorium is a swollen, modified germ tube tip, closely appressed to the cuticle. In many cases, owing to the retardation of apical elongation because of the tenacious adherence of the appressorium, the germ tube arches as shown in figure 2, *C, E, F, G, H, J*.

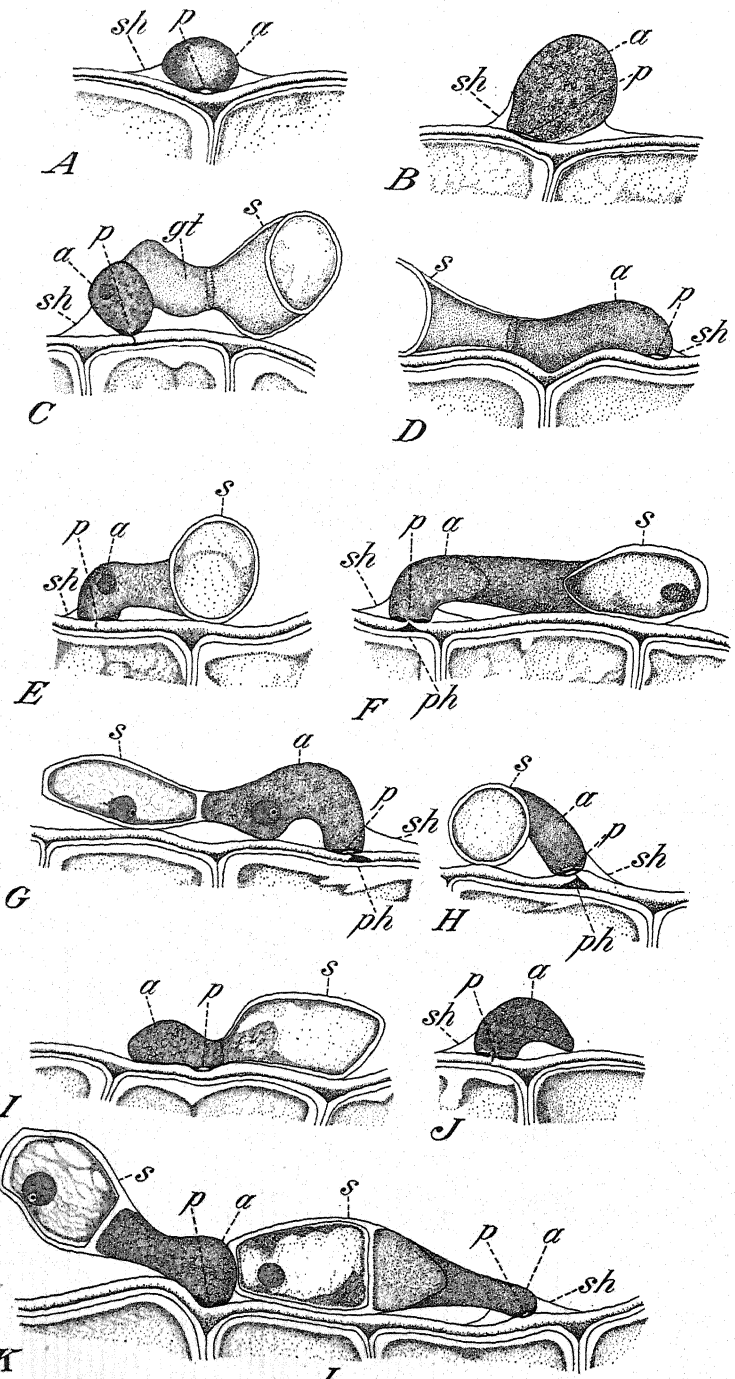


FIGURE 2.—Various stages of penetration from material fixed 20 hours after inoculation with isolates 22 and 17: *A*, 22 on Missouri Pippin; *B*, 22 on Fameuse; *C*, 22 on Yellow Transparent; *D*, 17 on Missouri Pippin; *E–G*, 22 on Fameuse; *H*, 22 on Yellow Transparent; *I*, 22 on Missouri Pippin; *J*, 22 on Fameuse; *K–L*, 17 on Missouri Pippin. Spore, *s*; germ tube, *gt*; appressorium, *a*; mucilaginous sheath, *sh*; pore, *p*; primary hypha, *ph*. × 1,620.

Mucilaginous Sheath

The appressoria were apparently held fast to the leaf surface by a mucilaginous sheath. The fixed remains of this structure were usually found in the cytological preparations (fig. 2, *A*, *B*). The sheaths, however, could not be demonstrated in connection with germinating conidia on glass slides by the dilute india-ink method, and they were not seen with certainty in the cleared-leaf preparations (*14*, *33*).

Circular Thickening and Pore

A circular, disklike area of thickening, usually about 6μ in diameter, occurred in the wall of the appressorium that was in contact with the leaf surface. In the middle of this structure was a circular, transparent, thin-walled, porelike area about 2μ in diameter. For convenience, these structures are subsequently referred to as the circular thickening and the pore (fig. 1, *A*, *B*, and fig. 2, especially *A*, *B*, *D*, *H*, *I*).

Infection Hypha

At or near the center of the pore, a minute infection hypha pierced directly downward or slightly obliquely through the cuticle (fig. 2, *C*, *E*, *G*, *J*). Such structures appeared in most of the 20-hour-old preparations in each isolate-variety combination. None was found in 14-hour preparations nor in 48-hour or older ones. In view of these facts and taking into account the stage of development in most 20-hour preparations, it is assumed that actual penetration began about 16 to 18 hours after inoculation, and that the infection hyphae disappeared or were not sufficiently stained to be seen shortly after infection had been established.

Primary Hypha

As soon as the infection hypha reached the epidermal cell wall it began to flatten into an irregularly shaped primary hypha (fig. 2, *C*, *F*, *G*, *H*). Further development of the fungus and the reactions of the underlying host tissues were different in the various isolate-variety combinations. Consequently, they will be discussed separately.

DEVELOPMENT OF INFECTION

A description of phenomena resulting from the different host-parasite reactions in the several isolate-variety combinations follows. The two combinations that favored aggressive parasitism are discussed first, and the reciprocal combinations, in which there was a strikingly lower degree of parasitism, are treated comparatively. The combination of each isolate with the resistant variety is discussed separately, because the host-parasite relations were decidedly different in the two cases.

AGGRESSIVE PARASITISM

Isolate 22 was aggressively parasitic on Yellow Transparent and 17 was equally aggressive on Fameuse. These infections were characterized by the vigorous development of the fungal hyphae, the formation of relatively thick stromata in the subcuticular position, prolific

sporulation of the parasite, and extreme impoverishment of the underlying host tissues, culminating in their death and collapse.

As previously described in the cleared-leaf study, the primary hyphae were at first single-celled and somewhat amoeboid or irregular in shape. A 24-hour-old preparation (fig. 3, *E*) shows that the primary hypha (*ph*) has expanded until it nearly covers the underlying epidermal cell and appears to contain a nuclear division figure, probably its first. A prominent nucleus remains in the appressorium (*a*). The underlying host tissues show no ill effects of fungal invasion. Another 24-hour-old infection (fig. 3, *B*) shows a young stroma developing beneath the thick cuticular layer in an epidermal hair socket. Many instances of penetration at the base of epidermal hairs were encountered.

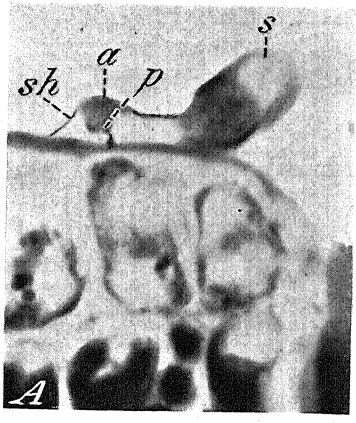
A median section of a 2-day-old lesion (fig. 3, *A*) illustrates the rapid proliferation of the primary hypha to form a multicellular stroma (*ps*), from which a slender stolonlike branch (*st*) is beginning to radiate. The fungal cells are uninucleate, the nuclei being comparatively large and prominent. These young fungal cells in the primary stroma, although appearing hyaline in cleared-leaf preparations, are filled with very dense cytoplasm which stained heavily in cytological preparations. In fact, in some preparations the cytoplasm is so dense that it masks the nuclei almost completely. The host nuclei in the underlying epidermal cells retain their normal shape and staining reaction. The cells, however, appear to be slightly plasmolyzed. This plasmolysis is attributed to the fixative, rather than to any deleterious fungal activity, because epidermal cells considerably removed from infected regions are similar in this respect.

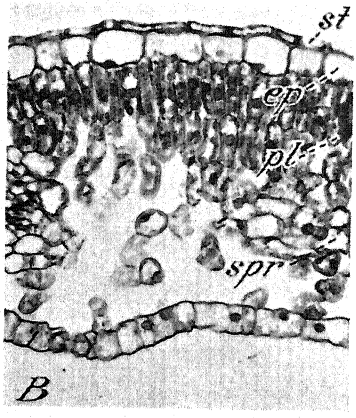
Throughout the incubation period, during which the fungus became firmly established and widespread in the subcuticular region, there was marked congeniality between the parasite and host. The fungus developed very rapidly and the underlying epidermal and palisade cells remained normal in appearance. The host tissues beneath a vigorously expanding stolonlike hypha in a 6-day-old infection (fig. 3, *F*) are just as normal in appearance as those beyond the area of infection. All nuclei retain their normal structure, staining reaction, and position. In addition, the palisade cells apparently contain the full complement of normal chloroplasts.

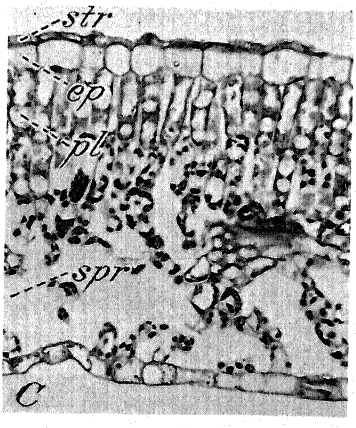
Coincident with the macroscopic appearance of the lesions, about 9 or 10 days after inoculation, impoverishment of the upper palisade layer appeared in the middle of the lesion. This depletion was manifested chiefly by the disappearance of plastids and by marked vacuolation as shown in plate 1, *D*.

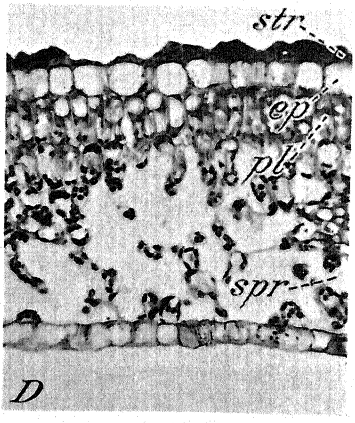
EXPLANATORY LEGEND FOR PLATE 1

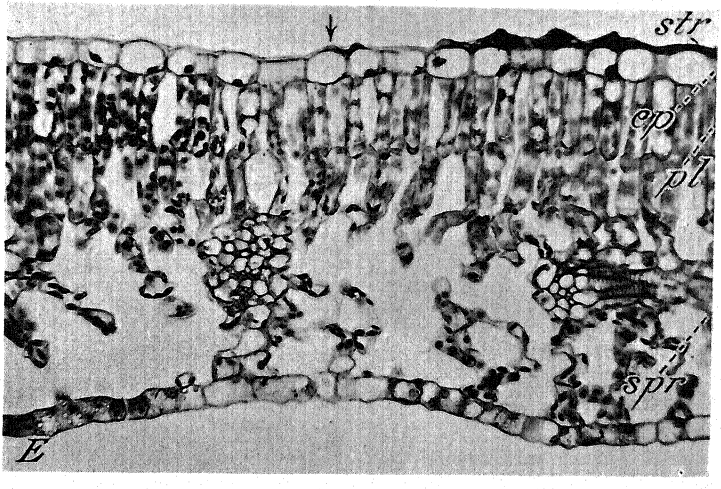
A, Isolate 22 on Yellow Transparent 20 hours after inoculation. The original photomicrograph, taken from the same lot of material as figure 2, *C*, was enlarged approximately × 2 with a photographic projector. Spore (*s*), appressorium (*a*), mucilaginous sheath (*sh*), infection hypha penetrating the unstained cuticle at the pore (*p*). × 800. *B*, Isolate 22 on Yellow Transparent 6 days after inoculation. Stolonlike hypha (*st*) near the periphery of lesion; underlying epidermis (*ep*), palisade (*pl*), and spongy parenchyma (*spr*) apparently normal. × 330. *C*, Isolate 17 on Fameuse 16 days after inoculation; young stroma (*str*) about midway between the center and the periphery of the lesion; upper epidermis (*ep*) and palisade (*pl*) impoverished; spongy parenchyma (*spr*) showing full complement of normal plastids. × 330. *D*, Isolate 17 on Fameuse 10 days after inoculation: Stroma (*str*) in the center of the lesion; upper epidermis (*ep*) and palisade layer (*pl*) vacuolate; spongy parenchyma region (*spr*) normal. × 330. *E*, Isolate 17 on Fameuse 16 days after inoculation: Peripheral stroma (*str*) above impoverished upper epidermis (*ep*) and palisade layer (*pl*); underlying spongy parenchyma region (*spr*) normal; arrow points to extreme peripheral hypha which lies just above the line of demarcation between impoverished and normal host tissues. × 330.



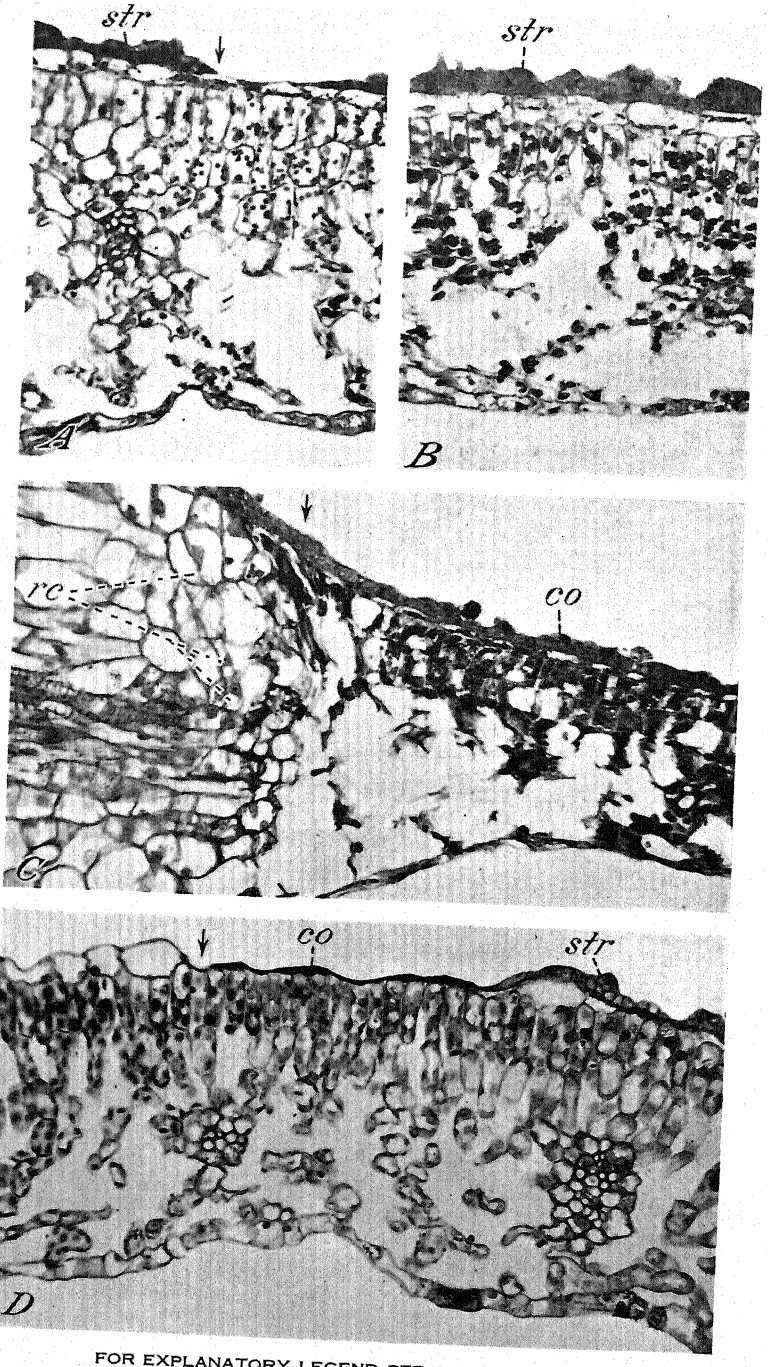








FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



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Impoverishment of the palisade region usually became coextensive with the lesion on the fourteenth or fifteenth day after inoculation. A small portion about midway between the center and the periphery of a 16-day-old lesion is shown in plate 1, *C*. The highly vacuolate upper palisade cells, which are practically devoid of plastids, appear in decided contrast to the spongy parenchyma cells below, which contain a full complement of apparently normal chloroplasts. The margin of a similar lesion (pl. 1, *E*) shows that the region of impoverishment coincides with or even extends a short distance beyond the area of fungal invasion.

Following impoverishment of the host tissues, death and collapse of the affected cells gradually ensued. First, the remaining contents of the cells lost their organized structure and became aggregates of small globules, which appeared to be homogeneous and stained heavily with safranine. These globules coalesced into larger irregularly shaped masses, and finally the cell walls collapsed. The cells later lost their identity completely. Progressive stages of this process are shown in plate 2, *A*, *B*, *C*. In *A*, death of cells is just beginning to occur and is manifested first in the epidermal layer. In the mesophyll tissues beneath, an arrow points to the line of demarcation between the region of extreme impoverishment on the left and that of death and incipient collapse on the right.

In plate 2, *B*, all of the mesophyll cells are dead and their protoplasts disorganized. Very few of the cell walls have collapsed, however, and they still retain their avidity for the acid stain. In *C*, an arrow indicates the margin of a totally collapsed area near the center of a 30-day-old lesion. Rhomboidal cells (*rc*), typical of wound reaction, are formed between the region of collapse and that of extreme depletion.

Injury or death of fungal cells followed the collapse of the host tissues beneath. In the two instances shown in plate 2, *A*, *B*, the subcuticular mycelium (*str*) is still alive while in *C* the mycelium at *co* has collapsed.

When the lesions attained the age of approximately 1 month, spread of infection practically ceased. The typical margin of an old lesion is shown in plate 2, *D*. The region of palisade cell depletion, which is indicated by an arrow, is coextensive with the area invaded by the fungus. The outpost hyphae, however, as well as the epidermal cells (*co*) beneath them, have collapsed. A narrow zone of fungus (*str*) a short distance back from the periphery remains alive. The occurrence of this zone of living fungal tissue is common, but not universal, and is often associated with the larger veins.

EXPLANATORY LEGEND FOR PLATE 2

A, Isolate 17 on Fameuse 21 days after inoculation. Living stroma (*str*) above collapsed upper epidermal layer; arrow points to line of demarcation between region of incipient collapse on the right and that of extreme impoverishment on the left. *B*, Isolate 17 on Fameuse 21 days after inoculation. All mesophyll cells are dead and beginning to collapse; fungal cells of stroma (*str*) still living. *C*, Isolate 17 on Fameuse 30 days after inoculation. An arrow points to the line of demarcation between the region of total collapse on the right and that of extreme impoverishment on the left; rhomboidal cells (*rc*) at the line of demarcation indicate cork formation; collapsed fungal stroma (*co*) associated with necrotic leaf tissue. *D*, Isolate 22 on Yellow Transparent 36 days after inoculation. Arrow indicates margin of lesion, which corresponds with the region of palisade impoverishment; peripheral hyphae and underlying epidermal cells have collapsed at *co*; a narrow zone of fungus (*str*) a short distance back from the periphery remains alive. All × 330.

INTERMEDIATE PARASITISM

In contrast to the manifestations of aggressive parasitism just described, the reciprocal isolate-variety combinations (22 on Fameuse and 17 on Yellow Transparent) showed a significantly lower degree. Fungal development was less vigorous, impoverishment of underlying host tissues was less severe, necrosis of host tissues was much less apparent, and conidia were not produced.

Further study of material from these intermediately parasitic combinations indicated that the host-parasite reactions differed from those of the aggressive combinations in degree rather than in kind. In young infections the cells of the mycelium were robust, normally developed, and apparently unharmed. The underlying host tissues were not noticeably affected until about 14 days after inoculation, when impoverishment of the upper palisade layer began to be evident. In older infections, the impoverished area usually became coextensive with the lesion and involved the whole mesophyll. Necrosis was usually confined to the epidermal layer immediately beneath the fungal stroma. Ultimately the epidermis and superimposed fungal stroma collapsed and lost their identity (pl. 3, *B*).

PHENOMENA OF RESISTANCE

Missouri Pippin was resistant to each of the two isolates, but the host-parasite reactions following infection by 22 were distinctly different from those following infection by 17.

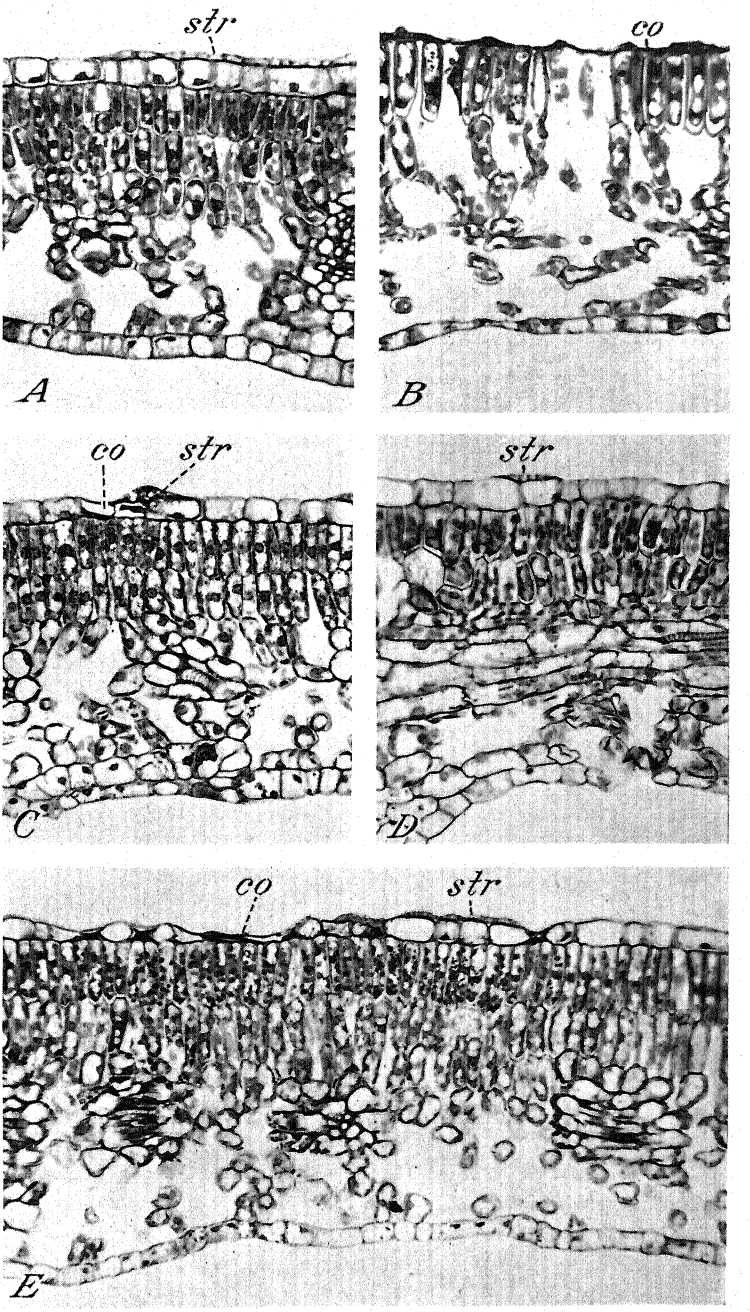
ISOLATE 22

The epidermal cells of this resistant variety were hypersensitive to attack by isolate 22, collapsing soon after the fungus became established beneath the cuticle. The primary hyphae (fig. 3, *D*, *ph*) of a 2-day-old infection is composed of several small cells, each of which contains one conspicuous nucleus. The appressorium (*a*) is still attached to the cuticle, and the pore (*p*) in its lower wall indicates the point of penetration, although the infection hypha cannot be seen. The epidermal cell (*de*) beneath the primary hypha has collapsed and the nuclei (*in*) in adjacent epidermal cells show signs of disorganization. The palisade cells beneath show no deleterious effects.

The fungus continued to spread feebly, however, sometimes forming a lesion 2 mm in diameter. Along the paths of the rather feebly developed hyphae scattered groups of epidermal cells succumbed. The fact that dead epidermal cells were frequently found near the margin of a lesion is further indication that the cells of the epidermis were highly intolerant of the parasite. A photomicrograph of the central part of a 16-day-old infection (pl. 3, *C*) shows a hyphal strand (*str*) in cross section, and beneath it are two collapsed epidermal cells (*co*). A similar view of a fungal strand in longitudinal section in a 24-day-old infection (pl. 3, *E*) shows that most of the epidermal cells (*co*) along the path of the fungus have died and collapsed.

EXPLANATORY LEGEND FOR PLATE 3

A, Isolate 17 on Missouri Pippin 24 days after inoculation. Longitudinal section of hyphal strand (*str*) near the center of the lesion; underlying mesophyll tissues apparently normal. *B*, Isolate 22 on Fameuse 30 days after inoculation. Fungal stroma and upper epidermis collapsed at *co*. *C*, Isolate 22 on Missouri Pippin 16 days after inoculation. Cross section of fungal strand (*str*) near the center of the lesion; two underlying epidermal cells collapsed at *co*. *D*, Isolate 17 on Missouri Pippin 16 days after inoculation. Cross section of fungal strand (*str*) near the center of the lesion; underlying leaf tissues normal. *E*, Isolate 22 on Missouri Pippin 24 days after inoculation. Longitudinal sections of fungal strand (*str*) near the center of the lesion; fungal cells and epidermis collapsed at *co*; underlying palisade region injured All × 330.



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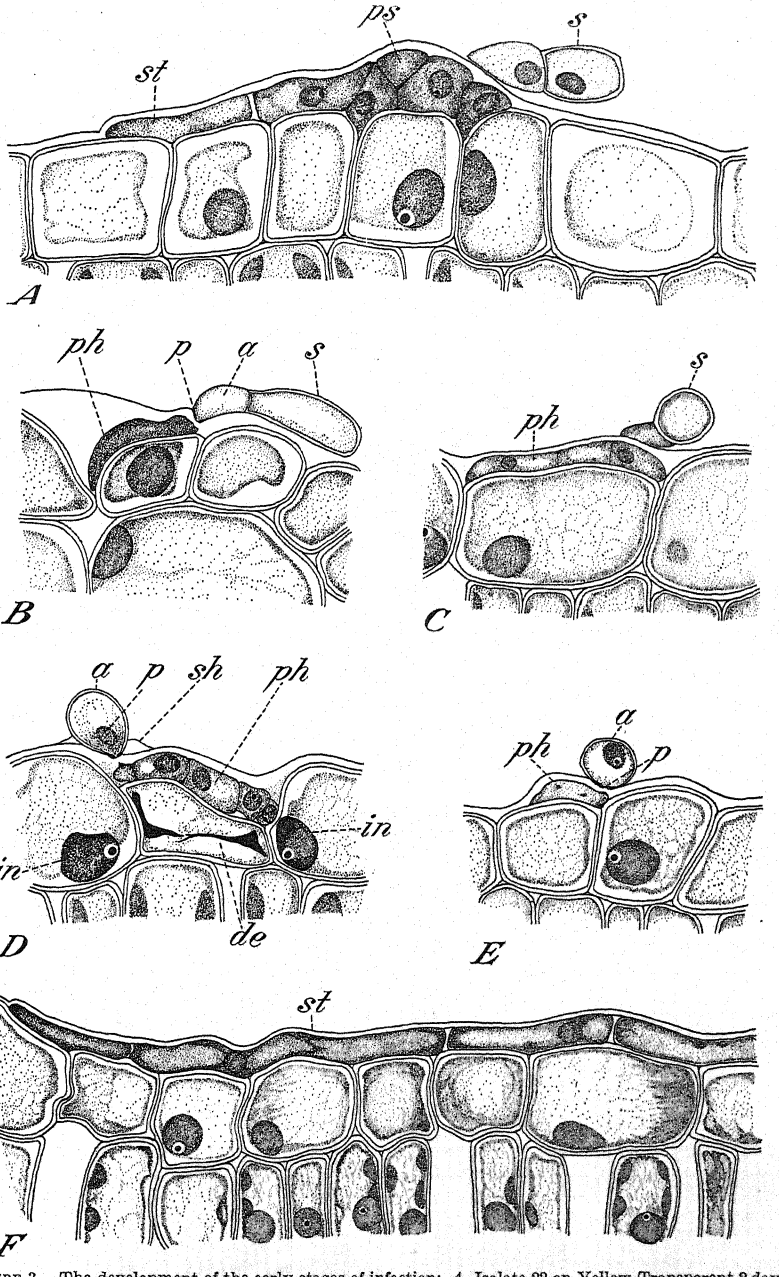


FIGURE 3.—The development of the early stages of infection: *A*, Isolate 22 on Yellow Transparent 2 days after inoculation; spore (*s*), primary stroma (*ps*), stolonlike hypha (*st*); underlying epidermal cells normal. *B*, Isolate 17 on Fameuse 24 hours after inoculation, showing hair-socket infection; spore (*s*), appressorium (*a*), pore (*p*), primary hypha (*ph*). *C*, Isolate 17 on Missouri Pippin 2 days after inoculation; spore (*s*), appressorium lost in sectioning, primary hypha (*ph*). *D*, Isolate 22 on Missouri Pippin 2 days after inoculation; appressorium (*a*), mucilaginous sheath (*sh*), pore (*p*), primary hypha (*ph*), epidermal cell collapsed at *de*, nuclei in adjacent cells at *in* injured. *E*, Isolate 22 on Yellow Transparent 24 hours after inoculation; appressorium (*a*), pore (*p*), primary hypha (*ph*). F, Isolate 22 on Yellow Transparent; stolonlike hyphal branch (*st*) at the periphery of a 6-day-old infection. All × 1060.

Although the underlying palisade cells showed very little depletion, they were apparently injured. The plastids remained and no abnormal vacuolation appeared. As indicated in the upper palisade layer of plate 3, *E*, and shown in detail in the drawing taken from a 16-day-old infection in figure 4, *E*, *ci*, aggregates of small globules appeared in the cytoplasm. These intracellular bodies have been found commonly in certain plant cells parasitized by various fungi and also in cells normally senescent. It is generally believed that they are probably disintegrative products of host cells (*24, p. 37*).

In the central portions of older lesions, where the mycelium was oldest, injured and sometimes dead fungal cells were found. In figure 4, *E*, the fungous cells, *ih* had become highly vacuolate and the one at *dh* was dead, whereas the cells *h* were apparently still robust and healthy. Observations of many similar cases lead to the interpretation that the hyphae live for some time directly superimposed upon dead epidermal cells, but finally they become injured and die.

Isolate 17

The young leaves of Missouri Pippin were decidedly unfavorable for isolate 17. The primary stroma developed very feebly, as shown in a 2-day-old infection in figure 3, *C*. The fungus, however, was able to push out long slender hyphal branches which sometimes involved an area of leaf surface 6 mm in diameter in one lesion. These hyphae were but sparsely branched, and robust secondary stromata were not found. A cross section of one of these hyphal strands near the center of a 16-day-old infection is shown in plate 3, *D*. The strand (*str*) is made up of about four hyphae and each cell appears to be empty. A longitudinal section of a hyphal strand from a 24-day-old infection is shown in plate 3, *A*. The fungal cells (*str*) are sparsely septate and nearly empty, except for inconspicuous nuclei. A drawing (fig. 4, *C*) taken from near the center of a 24-day-old infection shows the details of these host-parasite relations. The hyphal cells (*str*) are long and slender, and contain inconspicuous nuclei (*n*). The underlying host tissues here, as well as those shown in the photomicrographs, are apparently unharmed.

Relationship of the Parasite to the Epidermal Membranes of the Host

The differential staining of the cuticle and the outer wall of the epidermal cell afforded a means of studying the relationship of the apple scab parasite to these membranes. By treating fixed, sectioned material first with Delafield's haematoxylin and then with Sudan IV and mounting in glycerin, the walls of the host cells and of the fungus

Explanatory Legend for Figure 4

Figure 4.—Certain host-parasite reactions: *A*, Isolate 22 on Yellow Transparent 16 days after inoculation; advancing stolonlike hypha (*st*) cleaving its way between the cuticle (*c*) and epidermal cell wall (*w*), the fungus closely adherent to the epidermal cell wall even over the junction of two cells (*j*) where the cuticle is thickest. *B*, Isolate 22 on Yellow Transparent 16 days after inoculation; stolonlike hyphal branch (*st*) near the periphery of the lesion closely adherent to the epidermal cell wall even over the junctions of epidermal cells at *j* where the cuticle is thickest. *C*, Isolate 17 on Missouri Pippin 24 days after inoculation: cells of the stroma (*str*) in the central part of the lesion nearly empty except for small nuclei (*n*); underlying host tissues normal. *D*, *F*, *G*, Isolate 22 on Yellow Transparent 24 days after inoculation, showing successive stages of piercing of cuticle by conidiophores; cuticle (*c*), epidermal cell wall (*w*), conidiophore (*cp*), stroma (*str*), closely fitting collar (*cl*) formed by pierced cuticle about the base of the conidiophore. *E*, Isolate 22 on Missouri Pippin 16 days after inoculation; collapsed epidermal cells (*dc*) underlying a strand of fungus near the center of the lesion, dead fungal cell (*dh*), injured fungal cell (*ih*), apparently healthy cells (*h*), palisade cells slightly impoverished and showing globular inclusions at *ci*. *H*, Isolate 17 on Fameuse 30 days after inoculation; gaps (*g*) in the fungal stroma (*str*) bridged by the cuticle, impoverishment of underlying palisade cells shown by depleted plastids (*dp*) and thin cytoplasm (*cy*). All × 1,060.

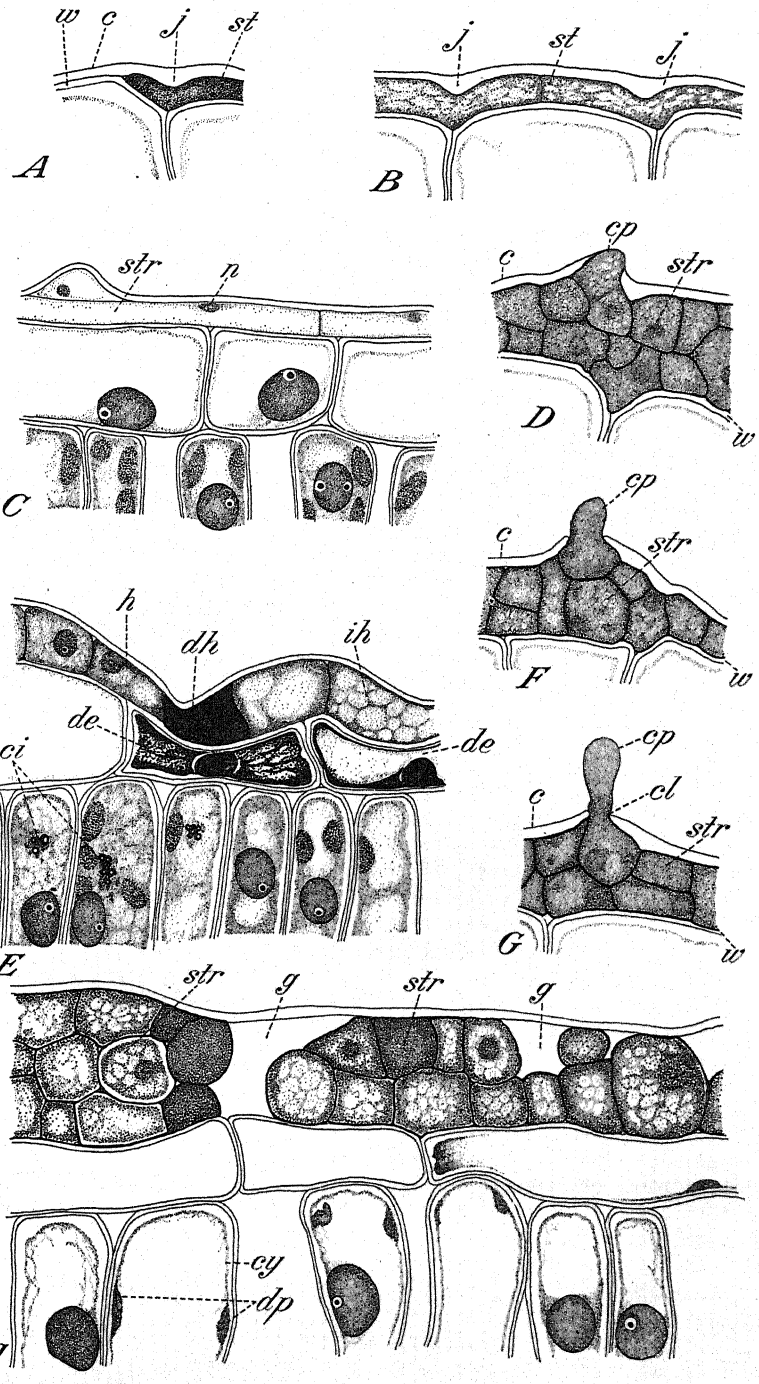


FIGURE 4.—Certain host-parasite reactions. (For explanatory legend see opposite page.)

stained bright blue, whereas the cuticle, being fatty in nature, stained red. The line of demarcation between the cuticle and the epidermal cell walls, in older leaves and over portions of the leaf where the cuticle was relatively thick, was quite sharp. In younger leaves the cuticle was decidedly thinner and was underlaid by a very thin layer that stained reddish purple, indicating that it contained a mixture of fatty and cellulosic materials. This subcuticular layer was sharply set off from the pure blue of the cellulose wall of the epidermal cells but graded off more gradually into the red of the cuticle proper. In older leaves the cuticle always was thickest over the junction of epidermal cells. It was too thin to permit accurate measurements. In the material studied there was no evidence of significant varietal differences in its thickness.

The fungus lay under the cuticle and in intimate contact with the cellulose walls of the epidermal cells. When advancing hyphae were viewed longitudinally, their tips appeared to be wedging or cleaving the cuticle from the epidermal cell wall. Even when passing over the juncture of two epidermal cells the hyphae dipped below the thickened cuticle. These phenomena are illustrated in two drawings made at and near the periphery of a 16-day-old lesion (fig. 4, *A*, *B*). Rarely, and only in preparations of the isolate 22-Missouri Pippin combination, has the fungus been observed to pass through these dentate thickenings of the cuticle. In such cases very small masses of red-staining material appear below the hyphae at the junction of cells.

Even in very old infections where the fungal stroma had become quite thick, the cuticle remained intact, except where conidiophores ruptured it, and acted as a protective covering for the fungus. In order to function in this manner, the cuticle must possess a high degree of elasticity and tensile strength. Indirect evidence to demonstrate these qualities is seen in figure 4, *H*, and the three drawings showing details of the way in which the conidiophores rupture the cuticle (fig. 4, *D*, *F*, *G*). In *H* the increase in size of the fungous cells created such a stress in the area between different stromata that the cuticle was locally ripped from the epidermal cell walls. Consequently, the cuticle bridged the gaps shown at *g*. This phenomenon was very common in older infections in aggressively parasitic isolate-variety combinations. In figure 4, *D*, an expanding conidiophore is stretching the cuticle outward. In *F* and *G* the cuticle has finally been penetrated and the conidiophore is free. The perforated cuticle, however, forms a closely fitting collar about the neck of the conidiophore at *cl*.

DISCUSSION

The results of the present investigation as they relate to spore germination and penetration of the parasite to the subcuticular position are in general agreement with those of Aderhold (*1*, *2*) and Wiltshire (*33*), though they differ in certain particulars. The emanation, in some cases, of the infection hypha from a germ tube or spore without the formation of a characteristically differentiated appressorium has been described in an earlier section of this paper (*14*). The development of the characteristic circular thickening and pore in the basal wall of the appressorium or its equivalent, as herein described, seems not to have been reported by the earlier investigators. In size

and form, the infection hyphae observed by the present writers differ much from those figured by Wiltshire (*33*), being so slender that they were very near the limit of visibility under the oil-immersion lens at a magnification of 1,425 diameters. These slender infection hyphae are very similar to those of *Gymnosporangium juniperi-virginianae* (*18*) and *Puccinia graminis* (*29*). While the possibility of occurrence of a minutely local solvent action on the cuticle by materials emanating from the fungus can scarcely be disproved by the technique employed, the present investigation yielded no convincing evidence that *Venturia inaequalis* exerts a solvent effect on the cuticle of its host. This finding is at variance with the conclusions of Wiltshire. There would seem to be greater probability of a similar action on the subcuticular layer, but the preparations studied by the writers did not show convincing evidence on this question.

As was pointed out in the introduction, parasitism of *Venturia inaequalis* is very distinctive, yet strikingly similar in many respects to that of many obligate parasites, such as certain of the rust and powdery mildew fungi. Notwithstanding the fact that the leaf-invading mycelium in its parasitic phase is limited to the subcuticular position, the fungus is able to derive its nourishment from the underlying host tissues so efficiently that it can develop a very substantial thallus and produce conidia abundantly. It is capable of inciting profound changes in host tissues many cells removed, in some cases leading to their death. As is common among the higher parasites, the species *V. inaequalis* is made up of many variant biotypes (*19*, *20*, *25*, *26*, *30*) with different pathogenic capabilities, some of which are able to live for many weeks in intimately balanced relations with the living tissues of a congenial host. Although in its parasitic phase the fungus is usually limited to subcuticular invasion, the efficiency of its nutrition seems to compare favorably with that of many fungi that freely invade the intercellular spaces or the living cells of the host. The mycelium in close contact with the cellulose walls of the epidermal cells is evidently very efficient in gaining nourishment through them. The subcuticular invaders of the type of *V. inaequalis* may therefore be regarded as a distinctive group among the higher parasites. The fact that they can readily be cultured in vitro and that at least one of them can be controlled genetically by breeding technique (*16*) makes them attractive subjects for further studies in the field of parasitism and disease resistance.

Though the cytological technique does not afford a basis for determination of the nature of resistance of apple varieties to *Venturia inaequalis*, it contributes to the foundation for further studies of this problem. It is noteworthy that spore germination and penetration of the cuticle of young leaves occurred freely, without reference to the isolate or the host variety used. These results, which are in general agreement with the work of Wiltshire (*33*), Johnstone (*9*), and Schmidt (*26*), indicate that resistance in young apple leaves is attributable primarily to relations of the fungus and materials emanating from the host tissues, rather than to mechanical barriers. Some possibilities as to the nature of similar host-parasite relations have been discussed by one of the writers (*18*, p. 591). The fact that substantially different reactions occurred when different incompatible host-isolate combinations were employed in the present work suggests the need for more

extended studies before these reactions can be satisfactorily characterized. It is noteworthy that in one of the combinations studied there was clear evidence of hypersensitiveness of the host, resulting in local necrosis of epidermal cells, while in the other, with another isolate on the same host, there was no evidence of hypersensitiveness.

The present study, in which two monoconidial isolates were employed on three apple varieties, has included four distinct types of host-parasite reaction. The observed variations in reaction are thought to be due partly to differences in the degree and partly to differences in the kind of phenomena concerned. It is to be expected that use of a wider range of biotypes of host and parasite would increase the range of variation in host-parasite relations. The knowledge that *Venturia inaequalis* is heterothallic and the availability of technique for breeding it (*17*) point the way to further studies of host-parasite relations, with genetically controlled isolates. Such work is in progress.

SUMMARY

The host-parasite relations of two monoconidial isolates of *Venturia inaequalis* on the leaves of three apple (*Malus sylvestris*) varieties were studied by cleared-leaf and cytological techniques. The six isolate-variety combinations employed showed four distinct types of host reaction, very susceptible, intermediate, and two types of resistant.

Spore germination, formation of appressoria or functionally equivalent structures, and direct penetration of the cuticle occurred in all cases, without being perceptibly influenced by isolate-variety combinations. A very tenuous infection hypha emanated from a minute pore surrounded by a circular thickening in the basal wall of the appressorium or its functional equivalent (sometimes a spore or germ tube), and pierced the cuticle without visible change in the thickness or staining reaction of the latter. The distal end enlarged to form a primary hypha when it reached the cellulose wall, and the fungus developed beneath the cuticle in close contact with the outer walls of the epidermal cells.

In very susceptible leaves host cells showed little abnormality until about 10 days after inoculation. Then progressive depletion of plastids and cytoplasm, attended by increasing vacuolation, began to appear in the upper palisade region at the center of the lesion. This impoverishment gradually spread throughout the area underlying the fungus, and was followed by necrosis. The fungus showed no apparent injury until the host cells had died. In leaves of intermediate susceptibility, the fungus developed less vigorously, impoverishment of the host cells was less rapid and severe, and little necrosis occurred. In resistant leaves, growth of the fungus was sharply restricted, the resistant variety employed showing hypersensitiveness to one isolate but not to the other. The resistance of young leaves is attributed primarily to relations of the fungus and materials emanating from the host tissues, rather than to mechanical barriers.

It is shown that in suitable isolate-variety combinations the fungus, though confined to the subcuticular position, is able to derive its nourishment efficiently from the underlying host tissues and to incite a wide range of pathological effects therein.

In its mode of penetration of the cuticle, diversity of isolate-variety reactions, and ability to live efficiently for many weeks in intimately

balanced relations with the living cells of a congenial host, *Venturia inaequalis* is considered to be strikingly similar to many of the so-called obligate parasites, and the subcuticular invaders of its type are regarded as a distinctive group among the higher parasites.

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DETERMINING THE SCLEROTIAL POPULATION OF SCLEROTIUM ROLFSII BY SOIL ANALYSIS AND PREDICTING LOSSES OF SUGAR BEETS ON THE BASIS OF THESE ANALYSES [1]

By L. D. LEACH, *assistant plant pathologist*, and A. E. DAVEY, formerly *associate in plant pathology, California Agricultural Experiment Station* [2]

INTRODUCTION

With most soil-borne plant pathogens it has been extremely difficult to determine the extent of survivial in commercial fields except by planting a susceptible crop and measuring the percentage of infection. The appearance of sclerotia as a stage in the life cycle of certain fungi provides a unit of the pathogen which can readily be separated from soils. King and Hope [3] have taken advantage of this fact to study the distribution of sclerotia of *Phymatotrichum omnivorum* (Shear) Duggar by washing samples of soil, taken with a soil auger, through screens of appropriate sizes. Rogers [4] constructed special equipment for the recovery of sclerotia of the same fungus from large soil samples.

DETERMINATION OF SCLEROTIAL POPULATION

ANALYSIS OF SOIL SAMPLES

In the summer of 1932 it was determined [5] that sclerotia of *Sclerotium rolfsii* Sacc. could be recovered from sugar-beet field soils by washing samples through a series of three soil screens of 10, 20, and 40 meshes to the inch. All sclerotia passed through the 10-mesh screen except occasional aggregates, which are rarely found in soil samples. The majority of sclerotia formed on favorable hosts were retained in the 20-mesh screen, but in certain samples large numbers were recovered on the 40-mesh screen. After the finer soil particles were washed through the screens the residue from each screen was flushed into a large white porcelain pan with sufficient water to make a depth of 2 cm. When this container was placed under a strong light it was comparatively easy to separate sclerotia from weed seeds and other extraneous material and to remove them with forceps.

To determine viability, the sclerotia recovered in this manner were surface-sterilized by immersion in bichloride of mercury 1–1,000 for 45 seconds, washed in sterile water, and then plated on potato dextrose agar and incubated at 30° C. The rapid growth of the fungus

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[2] The writers wish to express their appreciation to M. W. Gardner, plant pathologist and J. B. Kendrick, associate plant pathologist, University of California, for suggestions and criticism during these investigations.

[3] KING, C. J., and HOPE, C. DISTRIBUTION OF THE COTTON ROOT-ROT FUNGUS IN SOIL AND PLANT TISSUES IN RELATION TO CONTROL BY DISINFECTANTS. Jour. Agr. Research 45: 725–740, illus. 1932.

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mycelium necessitated the aseptic removal of blocks of agar surrounding germinated sclerotia at intervals of from 12 to 24 hours to prevent overgrowth of other sclerotia on the same plate. With some lots of sclerotia evidence was obtained that the bichloride of mercury was absorbed by sclerotia in amounts sufficient to prevent their germination.[6] In spite of the surface sterilization and ordinary laboratory precautions, contaminating fungi or bacteria from the interior of the sclerotia or from the air frequently interfered with the germination of sclerotia.

To eliminate these difficulties a revised method of determining viability has been used in all recent trials. Sclerotia are plated without chemical treatment on the surface of finely screened, unsterilized peat soil in Petri dishes. The soil is then thoroughly moistened and incubated at 30° C. for 5 days. The mycelium of *Sclerotium rolfsii* can be readily identified on the background of black soil (fig. 1). Germinating sclerotia and the mycelial colonies originating therefrom are removed from the surface of the plates each day during the incubation period.

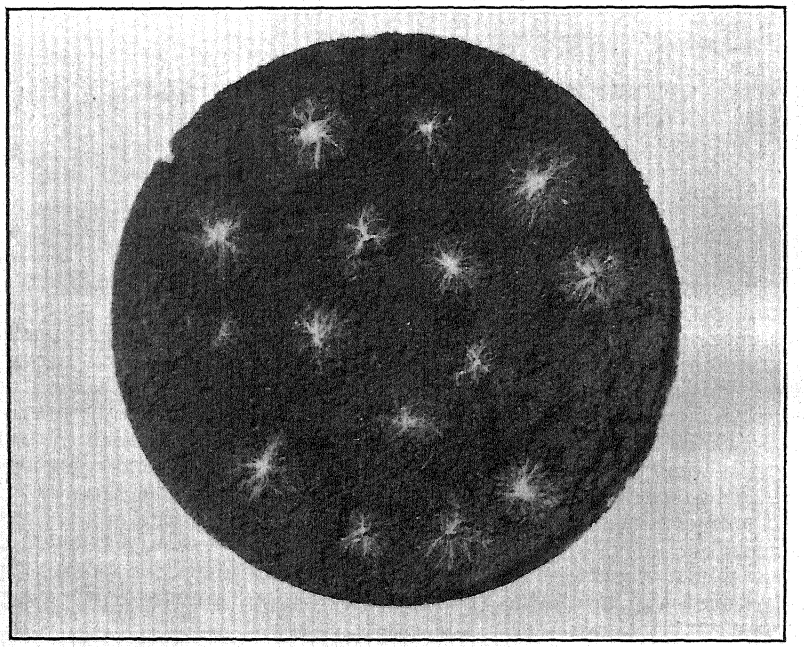


FIGURE 1.—Sclerotia on unsterilized peat soil after 30 hours' incubation at 30° C. Observe the well-developed mycelial colonies.

DISTRIBUTION OF SCLEROTIA IN SOILS

Studies on the distribution of sclerotia were undertaken as soon as the method of removing sclerotia had been perfected. This was done by excavation and observation in the fields and by counts of sclerotia recovered from soil samples representing different horizons.

It was determined that sclerotia may be formed on radiating

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hyphae at a distance of 20 inches or more from infected sugar beets (*Beta vulgaris* L.), but that the largest number occurred within 4 or 5 inches of the beet. From the soil samples studied it was estimated that the fungus in consuming a fully grown beet often produces between 10,000 and 20,000 sclerotia. The vertical distribution of sclerotia in the soil near infected sugar beets is shown in table 1.

TABLE 1.—*Vertical distribution of sclerotia in soil of sugar-beet fields*

Depth (inches)	Sclerotia per 200 g of soil			
	Field A		Field B	
	Number [1]	*Percent*	*Number* [1]	*Percent*
0–3	200	47.5	93	35.3
3–6	143	33.9	126	47.9
6–12	73	17.3	42	16.2
12–18	5	1.1	1	.3
18–24	1	.2	1	.3

[1] Average of 4 samples.

It is evident from table 1 that about 80 percent of the sclerotia occur in the upper 6 inches of the soil and that relatively small numbers of sclerotia are found below the 12-inch level.

The picture presented by a heavily infested sugar-beet field is that of a large number of small areas each containing an enormous population of sclerotia. These areas often coalesce because of the spread of mycelium from originally infected beets to adjacent plants. On the other hand, a small heavily populated area may be surrounded by an area containing few if any sclerotia. As the soil is disturbed by irrigation and cultural operations, the sclerotia gradually become more uniformly distributed. A certain degree of nonuniformity of distribution is maintained, however, by infection and multiplication of the fungus upon weeds or cultivated hosts.

METHOD OF SAMPLING INFESTED AREAS

During these investigations the plan for selecting soil samples has been modified as the work progressed. After several preliminary trials a permanently located plot 300 feet square (approximately 2 acres) was selected as a unit of area for the purpose of following the fluctuation of sclerotial population over a period of years. This area was sufficiently large to reduce the effect of small, severely infested spots. According to the first plan followed, soil samples were collected from nine stations equally spaced on the diagonals of the square. At each station, five cores were taken to a depth of 8 inches with a ⅞-inch soil tube. This method, although providing a picture of the irregular distribution of sclerotia, did not furnish a sufficient number of soil cores to provide an accurate estimate of the population of sclerotia. An improvement was made by collecting 10 soiltube cores in a circle with a radius of 10 feet at each of the 9 stations (fig. 2). The 10 cores from each station were washed through the screens as a composite sample.

When the main purpose is to determine the average survival of sclerotia within a given portion of the field it is possible to save con-

siderable time and effort with little loss of efficiency by combining all the samples collected from a given plot. After the soil is dried, pulverized, and thoroughly mixed it is passed through a Boerner sampler and four aliquot samples of 200, 400, or 800 g, depending on the degree of infestation, are selected. These four samples are washed through screens separately and the recovered sclerotia are plated to determine the percentage of germination.

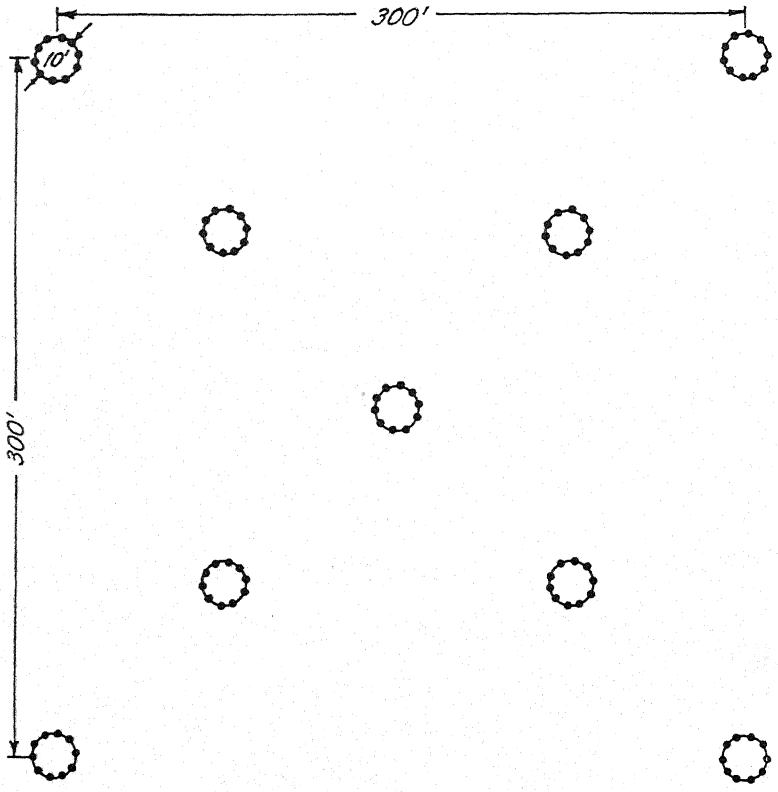


FIGURE 2.—Distribution of soil samples in a permanently located area; 10 soil cores are collected at each of the 9 stations.

TESTING THE ADEQUACY OF THE METHOD

The number of sclerotia obtained from the nine individual stations in several fields are shown in table 2. It would be highly desirable to apply statistical methods to the data and thus determine the reliability of the results. However, it can be readily seen that the distribution of numbers of sclerotia found in the individual samples is not normal and it is doubtful whether one is justified in analyzing such data statistically. In spite of this objection the standard error of the mean and the coefficient of variation have been determined for data from several fields.

TABLE 2.—*Number of sclerotia recovered in 1932 and 1934 from 200-g samples of soil in commercial fields*

Station No.	Field A, 1932 [1]	Field G, 1932	Field H, 1934	Field F, 1932	Field F, 1934
	Number	*Number*	*Number*	*Number*	*Number*
1	125	65	6	40	2
2	0	2	9	2	1
3	40	41	12	1	2
4	24	1	2	20	8
5	58	2	3	13	1
6	165	6	8	0	4
7	30	28	12	16	6
8	38	3	16	19	2
9	340	4	11	0	1
Mean	91.1	16.9	8.8	12.3	3.0
S. E. of mean	36.5	7.6	1.6	4.5	.9
	Years	*Years*	*Years*	*Years*	*Years*
Period elapsed since last beet crop	0	1	1	1	3
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Loss in last crop of sugar beets	70	75	50	50	50

[1] Number of sclerotia recovered from 200 g of air-dry soil.

TABLE 3.—*Number of sclerotia recovered from 400-g soil samples collected from 25 stations in sugar-beet fields F, H, and Q in 1934*

Number of sclerotia recovered and approximate position of indicated field-station samples															
Field F					Field H					Field Q					
*2	2	17	14	*3	*17	14	16	9	*13	*1	0	5		0	*1
9	*7	9	*2	3	29	*16	57	*6	43	117	*10	24		*0	0
2	3	*2	11	17	20	8	*23	29	13	12	34	*2		3	0
3	*11	2	*4	3	10	*24	10	*32	36	2	*36	2		*1	2
*4	3	11	42	*17	*24	27	4	51	*3	*6	62	5		3	*0

*Asterisks indicate the 9 samples that would have been collected in using the method illustrated in fig. 2. The first 3 columns under "Field Q" represent data from the part of the field that had been planted to sugar beets the previous year. See figure 3.

On the basis of 25 samples:
Field F: Mean, 8.0±1.86; coefficient of variation, 23.2 percent.
Field H: Mean, 21.4±1.92; coefficient of variation, 9.0 percent.
Field Q: Mean, 13.1±5.26; coefficient of variation, 40.1 percent.

On the basis of 9 samples:
Field F: Mean, 5.8±1.74; coefficient of variation, 30.0 percent.
Field H: Mean, 17.6±3.10; coefficient of variation, 17.6 percent.
Field Q: Mean, 6.3±3.87; coefficient of variation, 61.4 percent.

Standard error rather than probable error is shown after the sign ±.

In three fields the results obtained from 9 stations were compared with those from 25 stations distributed over the same area (table 3). In two of the cases, fields F and H, the infestation was uniformly distributed and the results obtained by taking the larger number of samples did not differ greatly from those obtained by the usual method. In contrast to the uniform distribution of sclerotia in the above-mentioned fields, field Q shows a distinct separation into areas of high and low populations. When the samples were taken a more uniform distribution was expected and the writers were at a loss to explain the results. Subsequently information was obtained which showed that one part of the field had been in sugar beets the previous season while the other part had been planted to a relatively resistant

crop. Figure 3 shows this field just before harvest. The portion of the field on the left suffered a loss of over 75 percent of the crop. (See data in table 3, first three columns under "Field Q"). The portion on the right indicated by soil samples as being lightly infested showed a loss of less than 20 percent at harvest time. The foregoing results indicate that in uniformly infested fields (fields F and H) the use of nine samples provides a fair estimate of the sclerotial population, whereas in a nonuniform field (field Q) the same number of samples is clearly inadequate. The use of 25 samples from each plot would seriously limit the scope of the survey. Permanently located plots have, therefore, been established only in uniformly infested fields, and since 1934 nine samples have been collected from such plots according to the plan shown in figure 2.

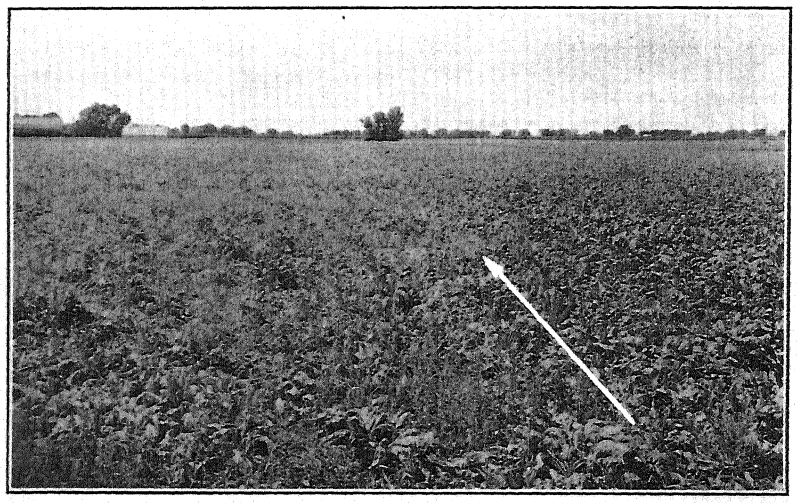


FIGURE 3.—Effect of replanting sugar beets in a field infested with *Sclerotium rolfsii* (field Q in table 3). The part of the field on the left of the arrow was in sugar beets the previous year (1933) and soil samples collected in 1934 before any beets were infected showed over 1,000 living sclerotia per square foot. Over 75-percent loss resulted. The part on the right was not in sugar beets the previous year and showed less than 100 living sclerotia per square foot. Less than 20 percent of the beets were lost by southern sclerotium rot.

NUMERICAL EXPRESSION OF SCLEROTIAL COUNTS

The population of sclerotia in field soils is expressed in this report in terms of sclerotia per square foot in the top 8 inches of soil (two-thirds of a cubic foot) and is derived in the following manner. The number of sclerotia recovered from a set of samples is multiplied by the quotient obtained by dividing the air-dry weight of the soil in grams into 25,100, the approximate weight in grams of two-thirds of a cubic foot of soil when the volume weight is 83 pounds per cubic foot. For example, if a total of 85 sclerotia is recovered from 10 soil samples whose total air-dry weight is 6,275 g, the average population of sclerotia per square foot is determined as follows:

$$85 \text{ (number of sclerotia)} \times \frac{25{,}100}{6{,}275} = 340 \text{ sclerotia per square foot, 8 inches deep.}$$

If the weight of air-dry soil is taken in pounds, the total weight is divided into 55.33 (pounds of soil in two-thirds of a cubic foot); and if in ounces, the total weight is divided into 885 (ounces of soil in two-thirds of a cubic foot).

Examples:

$$85 \text{ (number of sclerotia)} \times \frac{55.33 \text{ pounds}}{13.83 \text{ pounds}} = 340 \text{ sclerotia per square foot, 8 inches deep.}$$

or

$$85 \text{ (number of sclerotia)} \times \frac{885 \text{ ounces}}{221 \text{ ounces}} = 340 \text{ sclerotia per square foot, 8 inches deep.}$$

If the soil is considerably heavier or lighter than the standard (83 pounds per cubic foot) a correction can be made by multiplying by a factor more or less than 1.0. The indicated viable population is determined by multiplying the total population by the percentage of germination of the sclerotia as indicated by laboratory tests.

FLUCTUATION OF SCLEROTIAL POPULATIONS IN COMMERCIAL FIELDS

In table 4 are presented the total population of sclerotia and the viable population indicated by successive soil analyses from eight commercial fields over a period of from 2 to 5 years. The standard error of the mean for total population is also shown wherever the individual samples from each of the nine stations were screened separately. All other determinations were made from composites of all the soil collected from a given location. The enormous populations of sclerotia produced by *Sclerotium rolfsii* on sugar beets is illustrated by the results from fields A and B. These determinations indicate that with 70 percent of the beets infected in field A over 250,000,000 living sclerotia per acre were present at harvest time, and a 50-percent infection in field B resulted in the production of over 200,000,000 viable sclerotia per acre.

The effect of moderate populations of sclerotia on the infection of a crop of sugar beets is shown in fields C and D where indicated viable populations of 190 and 140 per square foot, respectively, each resulted in a loss of 20 percent of the sugar-beet crop. The populations of viable sclerotia produced in these two fields by the infestations were 2,190 and 3,120 respectively. The percentage of infection and the resultant population of sclerotia in field A, October 1936, are both comparatively low, but this can be accounted for at least partly as the result of a heavy application of nitrogen fertilizer.

In every case where severely infected crops of sugar beets were followed by nonsusceptible crops such as wheat and barley, or by winter crops such as peas, the sclerotia decreased rapidly by death or disintegration. On the other hand, when beans (susceptible) growing in infested soil were supplied with an abundance of surface irrigation water during the warm summer months the population was maintained, and, in some cases, was increased as shown in field B, November 1933; field E, December 1935; and field F, December 1935. The full effect of the increase was, in some cases, not observed until the second analysis following the bean crop as shown in field A, March 1934 and field C, March 1936.

TABLE 4.—*Indicated population of Sclerotium rolfsii sclerotia from successive soil analyses from eight commercial fields, 1932–36*

Field and time sampled	Crop grown	Total population per square foot	Standard error[1] of mean	Indicated[2] viable population
		Number		*Number*
Field A:				
September 1932	Sugar beets, 70 percent loss	9,840	±3,950	6,630
April 1933	Peas	3,060		1,620
November 1933	Beans	1,590		[3] 870
March 1934	Barley	2,890		1,050
August 1934	do	970		460
March 1935	do	440		190
March 1936	Fallow	390	±40	200
October 1936	Sugar beets, 5 percent loss	640	±180	170
Field B:				
December 1932	Sugar beets, 50 percent loss	7,130		4,810
July 1933	Peas	780		150
November 1933	Beans	1,050		[3] 410
March 1934	Peas	150		90
August 1934	Not known	200		80
July 1935	do	90		70
September 1936	Sugar beets, 5 percent loss	260	±170	190
Field C:				
1932	Sugar beets, 2 percent loss			
May 1933	Sugar beets	310		190
September 1933	Sugar beets, 20 percent loss	2,940		2,190
July 1934	Wheat	690		390
March 1935	Peas	420		350
December 1935	Beans	230		140
March 1936	Peas	350	±70	280
November 1936	Beans	60	±10	50
Field D:				
1933	Sugar beets, 5 percent loss			
1934	Wheat			
March 1935	Fallow	160		140
August 1935	Sugar beets, 20 percent loss	3,940	±1,900	3,120
March 1936	Wheat	2,370	±670	2,340
July 1936	do	1,210	±570	690
Field E:				
1931	Sugar beets, 25 percent loss			
September 1932	Wheat	1,080	±720	590
November 1932	do	1,650	±500	500
July 1933	do	220		60
October 1933	Beans	220		[3] 150
July 1934	Wheat	180		30
December 1935	Beans	490		[3] 370
July 1936	Wheat	570	±70	300
Field F:				
1931	Sugar beets, 50 percent loss			
September 1932	Wheat	2,250	±820	850
November 1932	do	2,240	±750	670
August 1933	do	1,020		300
May 1934	Fallow	500	±110	220
Do	do	360	±110	160
November 1934	Beans	50		40
December 1935	do	150		[3] 90
October 1936	do	200	±50	70
Field G:				
1931	Sugar beets, 75 percent loss			
September 1932	Barley	2,790	±1,250	1,370
July 1933	do	650		210
March 1934	Fallow	120		40
August 1934	Asparagus	0		0
July 1935	do	30		10
July 1936	do	[4] 380	±170	240
Field H:				
1933	Sugar beets, 50 percent loss			
March 1934	Fallow	1,130		650
May 1934	Planted to tomatoes	1,100	±190	920
Do	do	1,340	±120	1,080
March 1935	Fallow	160		90
August 1935	Lettuce	150		60
September 1936	do	100	±30	50

[1] Standard error of the mean for the total population (column 3).
[2] Total population times the percentage of germination.
[3] Some infection observed on beans; crops other than beets and beans showed little or no infection.
[4] Sclerotia produced on buried crowns of water grass.

An unusual case is presented in field G. The high population of sclerotia following a severe infestation on sugar beets was rapidly reduced during successive crops of barley in 1932 and 1933. Asparagus, a nonsusceptible crop, was planted in the spring of 1934, and samples collected in August of that year contained no sclerotia, indicating a very low survival. During 1935 and 1936, however, there occurred a significant increase. Observations showed that the increased population occurred, not upon the asparagus, but upon the decaying crowns of water grass (*Echinochloa crusgalli* (*L.*). Beauv.) which had been buried by cultivation preceding a surface irrigation during warm weather.

The differences between the results from successive sets of samples in the same field are usually comparatively large and in many cases are significant despite the relatively high standard error. Where successive determinations from the same field are available the uniformity of results lends weight to the accuracy of the estimates. Some idea of the differences in estimated population that are due entirely to errors of sampling can be secured by comparing the results from the analyses of September 1932 with those of November in the same year from fields E and F. Duplicate sets of samples collected at the same time are shown for field F, May 1934, and field H, May 1934. In each case the differences were well within the limits of differences required for significance as determined by the standard error computed from the results of individual samples.

The relation that exists between the percentage of infection on sugar beets and the population of viable sclerotia found in fields after harvest is shown in table 5. Determinations from 17 plots during 1934–36 show a correlation coefficient of 0.88 $\pm$0.056. It is apparent that the rate of multiplication is much greater in some fields than in others, but the causes of these differences have not been identified.

TABLE 5.—*Relation between the percentage of infection on sugar beets and the population of sclerotia in the soil after harvest*

Field No.	Infection on sugar beets	Indicated viable population of sclerotia per square foot	Field No.	Infection on sugar beets	Indicated viable population of sclerotia per square foot	Field No.	Infection on sugar beets	Indicated viable population of sclerotia per square foot
	Percent	*Number*		*Percent*	*Number*		*Percent*	*Number*
1	1	30	7	20	2,195	13	47	1,680
2	2	250	8	20	3,110	14	50	4,830
3	5	200	9	25	1,880	15	70	6,650
4	10	280	10	30	1,190	16	80	7,030
5	10	360	11	40	1,505	17	84	4,680
6	15	630	12	46	2,195			

PREDICTION OF LOSSES IN SUGAR-BEET FIELDS FROM COUNTS OF SCLEROTIA IN SOIL SAMPLES

Comparisons between the populations of viable sclerotia before planting as determined from soil samples and the percentage of infection occurring in sugar-beet crops had been made by the end of the 1935 season in 19 locations representing a variety of conditions.

There is considerable variation in the results from individual fields, but, in general, the percentage of infection was closely related to the original population of viable sclerotia (table 6). Analysis of the data indicated a correlation coefficient of 0.75 ± 0.10.

During the fall and winter of 1935–36 an attempt was made to determine whether this method of estimating populations of sclerotia could be extended to growers' fields and used as a basis for predicting the percentage of infection in a subsequent crop of sugar beets with reasonable accuracy. Sugar companies collected 354 soil samples from 38 growers' fields totaling 1,561 acres. Each sample consisted of

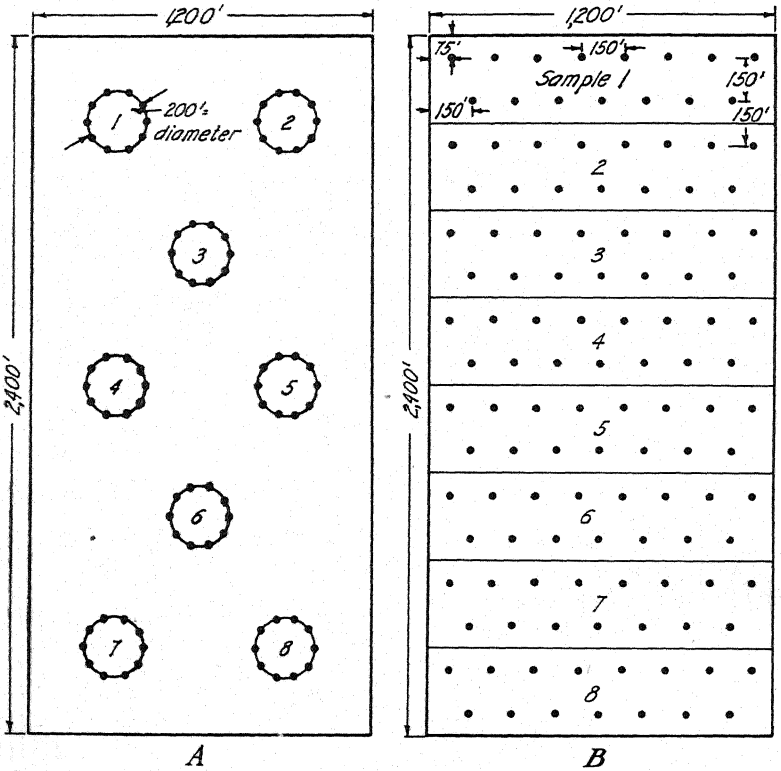


FIGURE 4.—Distribution of soil samples in commercial sugar-beet fields. *A*, Method used in the season of 1935–36. Ten soil-tube cores were collected at each station. *B*, Method used during the season of 1936–37. From 10 to 15 soil-tube cores were combined to form a sample.

10 soil-tube stabs to a depth of 8 inches collected from the perimeter of a circle from 100 to 200 feet in diameter. The sampled areas were uniformly distributed over the entire field intended for planting (fig. 4*A*). These samples were washed separately through screens, the sclerotia recovered, and their viability determined in the laboratories at Davis. Reports covering the indicated population for each sampled field were supplied to the companies. Several fields that had an indicated population of more than 100 viable sclerotia per square foot were rejected for planting to sugar beets for the 1936 season, since previous results had shown that such fields usually suffered a loss of at least 10 percent of the crop.

TABLE 6.—*Relation between the population of sclerotia of Sclerotium rolfsii before planting and the percentage of infection on sugar beets in the indexed area*

[Data collected 1932 to 1935]

Field No.	Indicated viable population of sclerotia per square foot	Infection on sugar beets	Field No.	Indicated viable population of sclerotia per square foot	Infection on sugar beets	Field No.	Indicated viable population of sclerotia per square foot	Infection on sugar beets
	Number	*Percent*		*Number*	*Percent*		*Number*	*Percent*
1	30	10	8	145	20	15	565	40
2	50	4	9	190	25	16	565	64
3	50	8	10	200	20	17	1,380	69
4	70	5	11	215	[1] 42	18	2,070	79
5	75	8	12	220	25	19	2,510	69
6	90	8	13	375	21			
7	95	[1] 35	14	450	50			

[1] Percentage of infection increased by high water table.

Prior to the date of harvest, disease counts were made by examining from 2,000 to 3,000 beets in each area of the field from which a sample was taken. The average percentage of disease for the field was calculated from the sum of all counts in that field.

In table 7 the population of viable sclerotia is shown for each sampled field along with the percentage of infection observed on the date of counting. Because numerous observations have shown that the percentage of infection increases rapidly during July and August, the disease counts have been placed on a comparable basis by converting them to an estimated percentage of infection as of August 1. This was done by applying the rate of increase indicated by an average mortality curve which was secured by counting, at intervals of 10 days or 2 weeks, the increase in number of diseased plants in replicated plots in 12 commercial fields during three growing seasons.

Analysis of the data from commercial fields (table 7) indicates a correlation coefficient of 0.85 ± 0.049 between the indicated population of viable sclerotia (column 2) and the percentage of infection on the subsequent crop (column 3). When the values for percentage of infection were converted to estimates as of August 1, the correlation coefficient (columns 2 and 4) was found to be 0.65 ± 0.10. The percentage of infection in field No. 21 was obviously increased by the excessively high water table. Analysis of the data with field 21 omitted showed a correlation coefficient of 0.86 ± 0.048 between the sclerotial population and the percentage of infection determined by counts (column 3) and of 0.77 ± 0.074 between the sclerotial population and the estimated percentage of infection as of August 1 (column 4).

In the same way the number of sclerotia recovered from each of 293 individual samples was compared with the percentage of infection in the same portion of the field from which the sample was taken. The correlation coefficient was found to be 0.52 ± 0.043 and the standard error of estimate 9.82 percent.

Analysis of the results from individual fields reveals the following information:

(1) As anticipated from previous results, all fields showing less than 100 sclerotia per square foot produced losses of less than 10 percent.

(2) Of the six fields yielding no sclerotia in samples, three showed no infection on beets; the other three showed less than 1 percent infection.

(3) All fields showing more than 200 sclerotia per square foot contained more than 15 percent infection.

(4) The percentages of diseased plants in areas affected by seepage or in those with excessively high water table were much greater than in normal fields with similar populations of sclerotia.

(5) Fields containing localized areas of severe infestations deviated widely (either higher or lower) from the anticipated level of infection.

(6) In two cases a below-average incidence of disease was associated with applications of nitrogen fertilizers.

TABLE 7.—*Relation between fungus populations as determined by analyzing soil samples and the incidence of subsequent infection as measured by field counts, season 1935–36*

Field No.	Viable sclerotia per square foot (average for field)	Infection on subsequent crop	Estimated infection as of Aug. 1	Field No.	Viable sclerotia per square foot (average for field)	Infection on subsequent crop	Estimated infection as of Aug. 1	Field No.	Viable sclerotia per square foot (average for field)	Infection on subsequent crop	Estimated infection as of Aug. 1
	Number	*Percent*	*Percent*		*Number*	*Percent*	*Percent*		*Number*	*Percent*	*Percent*
1	0	0.0	0.0	12	10	[1] 5.2	[1] 7.2	23	40	5.0	7.1
2	0	.0	.0	13	10	[1] 6.9	[1] 4.1	24	80	5.8	11.5
3	0	.0	.0	14	20	2.2	1.3	25	110	11.0	15.4
4	0	.5	1.0	15	20	2.2	1.4	26	130	[2] 3.0	[2] 1.7
5	0	.7	.4	16	30	2.0	1.2	27	140	12.9	9.0
6	0	.8	1.7	17	30	3.1	6.8	28	160	[2] 4.8	[2] 2.8
7	<10	.3	1.1	18	30	[1] 2.7	[1] 1.7	29	350	30.0	21.0
8	<10	.7	.4	19	30	3.3	5.0	30	550	15.4	21.6
9	<10	.7	.6	20	30	[2] 4.5	[2] 2.7	31	950	21.4	15.0
10	10	[1] 3.6	[1] 2.0	21	30	[3] 8.6	[3] 25.8	32	1,190	[1] 32.0	[1] 22.4
11	10	[1] 4.5	[1] 6.4	22	40	1.7	1.4				

[1] Field contained localized areas of high infestation.
[2] Nitrogenous fertilizers applied.
[3] Percentage of infection increased by high-water table.

The results from the individual soil samples showed a lower correlation with the incidence of disease in a particular portion of the field than did the field averages. One-half of the samples yielding no sclerotia were, however, associated with areas showing less than 0.5 percent infection, while three-fourths (15 out of 20) of the samples yielding more than 10 sclerotia (approximately 250 per square foot) were associated with areas showing more than 10 percent infection. In several cases the incidence of disease varied widely from the infestation indicated by the individual sample. This may have been due to error of sampling or to failure to locate accurately the sampled area when disease counts were made.

In view of the foregoing results a revision of the method of selecting soil samples was instituted during the season of 1936–37. Instead of collecting 10 soil-tube stabs from each of 10 or more portions of the field (fig. 4, *A*) single stabs are taken in straight rows across the field at intervals of 40, 50, or 70 yards depending on the intensity of sampling desired. The soil from 10 to 15 stabs (approximately 3 pounds) is combined to form a single sample and is screened as a unit. By staggering the location of the stabs in adjacent rows, a very complete sampling of a field can be secured (fig. 4, *B*). This method may tend to obscure the occurrence of small localized areas of infestation but is expected to greatly improve the reliability of the field average.

SUMMARY

A reasonably accurate approximation of the population of sclerotia of *Sclerotium rolfsii* can be secured by the examination of soil samples collected from growers' fields. The soil samples are washed through a series of three screens of 10, 20, and 40 meshes to the inch. Sclerotia are recovered from the residue and their germinability determined by incubation at 30° C. on the surface of unsterilized peat soil in Petri dishes.

In sugar-beet fields approximately 80 percent of the sclerotia occur in the upper 6 inches of soil and less than 2 percent of the sclerotia are more than 12 inches deep. Soil samples are, therefore, taken to a depth of 8 inches in undisturbed soils.

The population is expressed as the number of viable sclerotia per square foot of soil to a depth of 8 inches, the depth of sampling, and is computed by methods described in this paper. The information obtained in this manner provides a means of estimating the survival of *S. rolfsii* under different crop rotations and cultural conditions.

The fluctuation of sclerotial population has been followed in permanently located areas within 17 growers' fields over periods of from 2 to 5 years. Results from eight of these fields are presented. In each area 10 soil-tube cores were collected at each of nine stations on the diagonals of a square approximately 300 feet on a side.

Results indicate a relatively high correlation between the population of viable sclerotia, determined before planting, and the percentage of infected sugar beets in the same area. The number of sclerotia in the soil increased in proportion to the percentage of infection. Following 50 percent infection of beets, as many as 5,000 sclerotia per square foot have been found. Cropping infested fields with nonsusceptible crops such as wheat or barley, or with winter crops such as peas, results in rapid reduction of the sclerotial population. Susceptible crops, such as beans, when supplied with abundant surface irrigation during the summer months usually maintain the sclerotial population at a moderately high level.

During the seasons of 1932 to 1936 soil samples were collected from 51 fields or portions of fields that were subsequently planted to sugar beets. Fields with more than 200 viable sclerotia per square foot invariably showed more than 15 percent loss of sugar beets, while those with less than 100 per square foot usually showed less than 10 percent loss. This method of estimating the sclerotial population may be used to eliminate severely infested fields in advance of planting.

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THE EFFECTS OF TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS UPON THE PHOTOPERIODIC RESPONSES OF SOME OF THE HIGHER PLANTS[1]

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INTRODUCTION

Ever since the demonstration of the principle of photoperiodism by Garner and Allard in 1920 (*1*),[2] it has been believed by some workers that there are certain plants which will blossom only in a long- or a short-day environment. That is, it is considered that photoperiod has a specific effect upon the induction of the flowering state which is possessed only by this environmental condition. This view has persisted in spite of the fact that it has been known for a considerable time that celery (*9*), beets (*10*), lettuce (*11*), and stocks (*4*), which give responses to photoperiod, have been induced to blossom by "chilling" and that soybeans do not give the expected reaction to length of day when growing at high temperatures (*3*).

It has been briefly reported that the photoperiodic responses of a number of plants are modified by temperature (*7*). Since that report was made further trials have been completed, giving a total of more than 40 species and varieties which show altered reactions to length of day when growing at different temperatures. This list includes plants which are commonly considered to be specifically photoperiodic in their reactions. The present report is a presentation of the observations upon which these preliminary statements are based.

MATERIALS

A larger variety of plants was grown than was needed to test the effect of environmental treatments upon blossoming, as it was desired to secure samples from blossoming (reproductive) and nonblossoming (vegetative) plants to use in further studies of the obvious relation between the anatomical condition of the plant and its flowering state (*12*), especially the characteristics of the phloem (*8*). The list includes representatives of more than 40 families, 80 genera, and 120 species and varieties. The families and species follow:

Gramineae: *Zea mays, Holcus sudanensis, H. sorghum, Panicum miliaceum, Phalaris arundinacea, Phleum pratense, Avena sativa, Triticum aestivum, Secale cereale, Hordeum vulgare, Poa bulbosa, P. pratensis, Agropyron repens.*
Liliaceae: *Asparagus officinalis.*
Moraceae: *Cannabis sativa.*
Urticaceae: *Urtica dioica.*
Polygonaceae: *Fagopyrum esculentum.*
Chenopodiaceae: *Chenopodium album; Beta vulgaris, Spinacia oleracea.*
Amaranthaceae: *Amaranthus retroflexus, A. graecizans.*
Aizoaceae: *Tetragonia expansa.*

[1] Received for publication August 19, 1937; issued May 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 677.

Portulacaceae: *Portulaca grandiflora.*
Caryophyllaceae: *Dianthus plumarius.*
Ranunculaceae: *Delphinium cultorum.*
Capparidaceae: *Cleome spinosa.*
Cruciferae: *Thlaspi arvense, Capsella bursa-pastoris, Brassica oleracea, B. rapa, B. pekinensis, B. alba, Matthiola bicornis.*
Crassulaceae: *Bryophyllum proliferum.*
Leguminosae: *Phaseolus vulgaris, P. coccineus, Soja max, Medicago sativa, Melilotus alba, M. dentata, Trifolium repens, Baptisia australis, Mimosa pudica.*
Geraniaceae: *Pelargonium hortorum.*
Oxalidaceae: *Oxalis corniculata.*
Tropaeolaceae: *Tropaeolum majus.*
Euphorbiaceae: *Euphorbia pulcherrima, Ricinus communis.*
Balsaminaceae: *Impatiens balsamina.*
Malvaceae: *Althaea rosea, Gossypium hirsutum.*
Violaceae: *Viola tricolor.*
Begoniaceae: *Begonia semperflorens.*
Onagraceae: *Clarkia elegans, Oenothera pratensis, Fuchsia hybrida.*
Umbelliferae: *Daucus carota, Petroselinum hortense, Apium graveolens.*
Plumbaginaceae: *Plumbago capensis, P. indica.*
Apocynaceae: *Vinca minor.*
Asclepiadaceae: *Asclepias syriaca.*
Convolvulaceae: *Ipomoea purpurea, I. batatas.*
Polemoniaceae: *Phlox paniculata.*
Verbenaceae: *Verbena hybrida.*
Labiatae: *Salvia splendens.*
Solanaceae: *Solanum tuberosum, S. pseudocapsicum, Nicotiana affinis, N. sanderae N. tabacum, Datura stramonium, Petunia hybrida, Salpiglossis sinuata.*
Scrophulariaceae: *Antirrhinum majus.*
Plantaginaceae: *Plantago major.*
Acanthaceae: *Beloperone guttata.*
Cucurbitaceae: *Cucumis sativus, Luffa cylindrica.*
Compositae: *Cichorium intybus; Lactuca sativa; Chrysanthemum morifolium; C. maximum; Anthemis cotula; Rudbeckia laciniata; Helianthus maximiliani; Cosmos bipinnatus; C. sulphureus; Aster novae-angliae; Tagetes erecta; Gaillardia amblyodon; Cirsium arvense; Solidago canadensis; Sonchus oleraceus.*

More than one variety of a single species was grown in the following cases: Four corn, two timothy, three oats, two wheat, two rye, two barley, two sugar beet, two turnip, four soybean, two morning-glory, two potato, two tobacco, two cucumber, and two orange cosmos.

A minimum of four plants of each variety was used as a unit, for each treatment employed. Larger numbers were commonly grown. The tests of the more important species were repeated two to four times.

METHODS

Duplicate sets of plants were grown in two greenhouses during the winter of 1936–37, one house being carried at a minimum (night temperature) of 70° F. and the other at 55°. These temperature treatments will be referred to as the "warm" and "cool" environments. There was little variation from these temperatures during the daytime until late spring. After April 1 the cool house would sometimes be warmer during the daytime but to no great extent until after May 1, owing to the marked absence of high temperatures in early 1937. Outdoor maximum temperatures exceeded 60° on only 4 days until May 1 and 70° only twice before May 21. Minimum temperatures were above 55° on only 2 days prior to May 29. Plants from the cool house were transferred to out-of-door temperatures a little after danger from frost was judged to be passed, May 26.

A limited series of plants was grown during the winter season at an intermediate temperature of about 63° F.

Half of each lot of plants in the warm house and in the cool house was placed in a long-day and half in a short-day environment. The long-day condition was provided by ordinary electric lights, burning from before sunset until midnight. These delivered from 30 to 80 foot-candles, as measured by a Weston photometer, to the place where the plants were situated. Their use was discontinued May 28. The short-day treatment was the normal winter day from October 15 until March 1. Beginning on that date the light exposure was artificially shortened to approximately 9 hours.

Except as otherwise noted, the plants were grown in soil and watered according to greenhouse practice.

Other environmental treatments which were employed on a smaller number of varieties were bank-sand culture (low nitrogen), partial defoliation (fig. 1), shading (light intensities of 10 to 15 foot-candles), girdling, and chilling. Many plants were shifted from one environment to another at different stages in their development and others were grown with different branches or parts of the plant in the long- and short-day environments.

OBSERVATIONS

TEMPERATURE AND PHOTOPERIOD

The time factor should be carefully considered when determining the photoperiod classification of a plant. An inspection of the literature on photoperiodism shows a general tendency to call a plant a long- or a short-day one if blossoming is merely hastened by added light or by a shortened daily light exposure. In the present trials, in which different temperatures were employed, the time required to flower was largely ignored and the periodism of a plant was determined by whether the reproductive characteristics were readily induced by a treatment, even though actual blossoming was at a considerably later time (fig. 2).

Among the more than 40 species and commercial varieties of plants that give unlike responses to length-of-day environments when grown in different temperatures, there are a number which are of special interest. For instance, poinsettia, which is habitually considered to be a short-day plant, failed to blossom in short days when grown at a minimum temperature of 70° F. from September until April (fig. 3). On the other hand, 60 percent of the 20 plants which were given a long-day treatment blossomed at a temperature of 55°. Plants in an intermediate temperature of 63° to 65° gave the anticipated response of short-day blossoming and long-day vegetative growth.

This influence of temperature upon photoperiodism is not confined to short-day plants, as is demonstrated by the results with *Rudbeckia laciniata*. This long-day plant has consistently remained a rosette and failed to produce stems in previous short-day experiments (temperatures of 60° to 65° F.) (*6*). On the other hand, when grown at 55° it not only produced stems, but these formed blossom buds in the short-day treatment, after a relatively long time—a definite refutation of the commonly reported habits of this plant (figs. 4 to 7). The long-day cool plants produced abnormal blossoms (fig. 5), but with

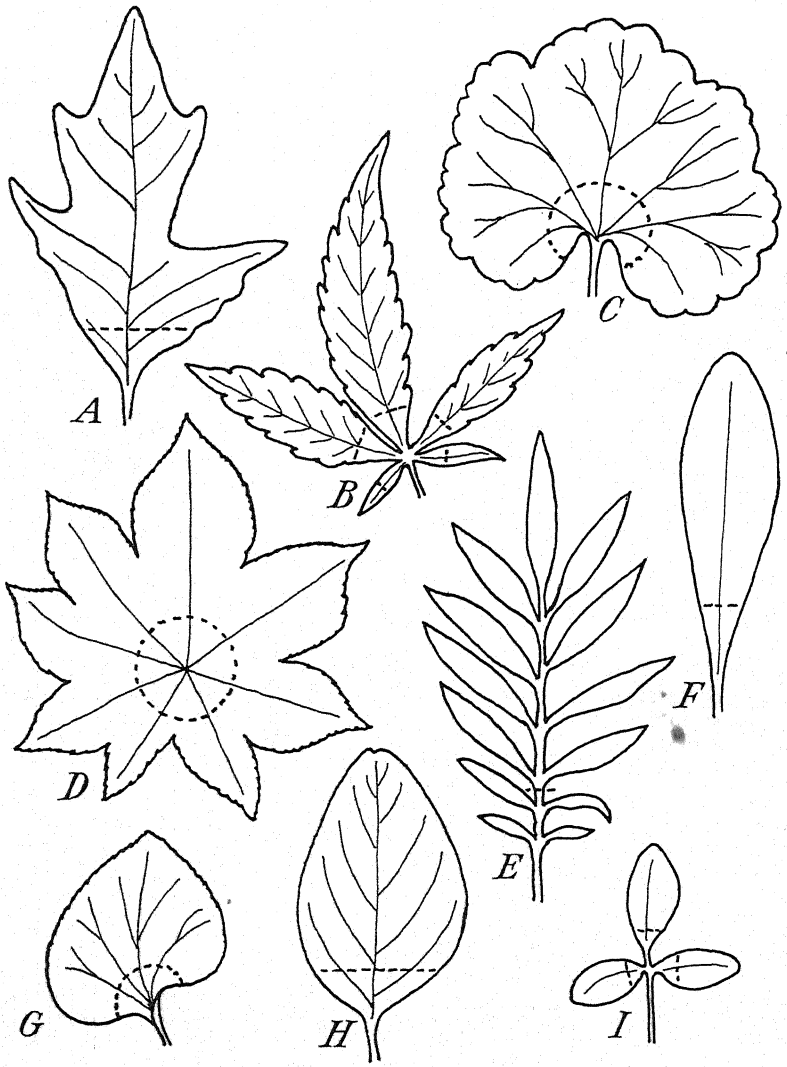


FIGURE 1.—Diagram of leaf outlines to illustrate the "defoliation" method (dotted lines): *A*, Poinsettia; *B*, hemp; *C*, geranium; *D*, castor-bean; *E*, marigold; *F*, stock; *G*, begonia; *H*, pigweed; *I*, sweetclover.

warmer temperatures became more like the usual type. The sow-thistle also remained as a rosette, in the short-day warm house, but produced a typical stem and fruited in the short-day cool house.

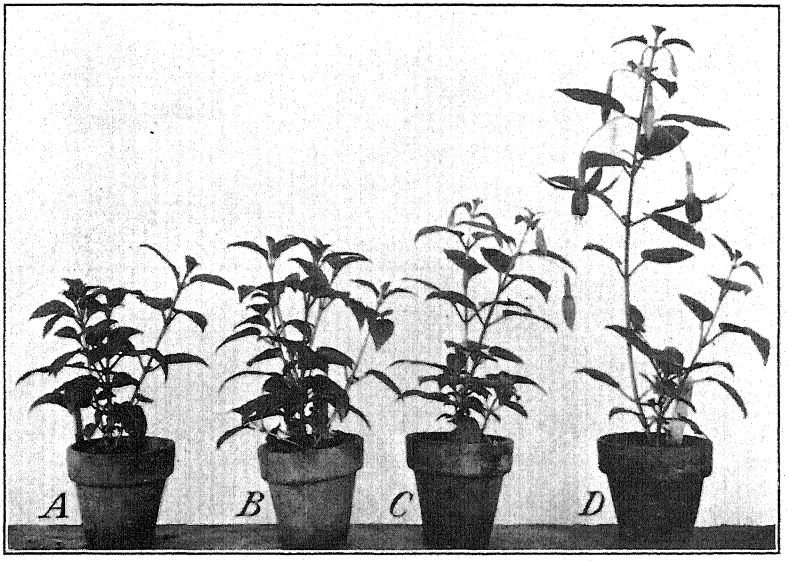


FIGURE 2.—Fuchsia blossomed in all environments but after different lengths of time. Such a variety should be classified as indeterminate and not as long-day: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day.

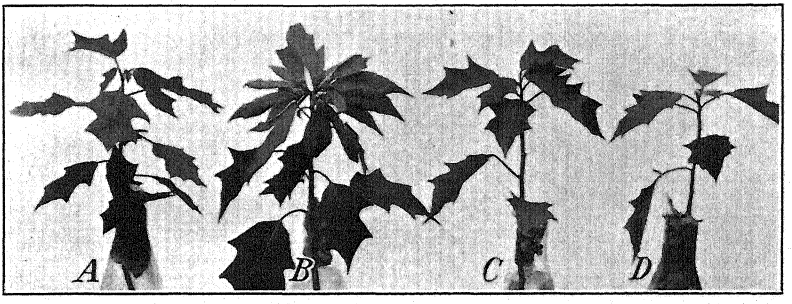


FIGURE 3.—Tips of poinsettia plants grown in different temperatures, all in short days: *A*, 70° F. minimum (vegetative); *B*, 63° (flowering); *C*, 55° (vegetative, slowly) *D*, changed from 65° to 70° (flowers abscissed).

Klondike cosmos (a "specifically short-day plant") did not blossom in the short-day warm house. In the cold treatments it grew very slowly, the long-day plants showing as much tendency to blossom as did the short-day plants (figs. 8 and 9).



FIGURE 4.—Temperature affects blossoming of rudbeckia: *A*, Photographed February 8; *a*, cool, short-day; *b*, cool, long-day; *c*, warm, short-day; *d*, warm, long-day; *e*, changed warm, long-day to warm, short-day. *B*, Photographed April 15; *a*, cool, short-day; *b*, cool, long-day. The production of shoots in cool, short days (*A*, *a*) is in striking contrast to the rosettes (*A*, *c*) in warm, short days (*6*). This same condition occurs with sow-thistle and with chicory (fig. 25).

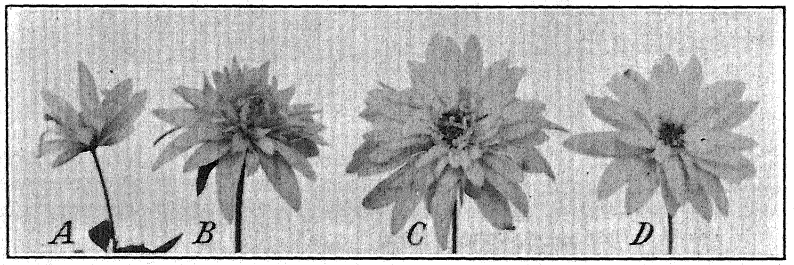


FIGURE 5.—*Rudbeckia* blossoms: *A*, Warm, long-day to cool, long-day; *B*, cool, long-day; *C*, cool, long-day to warm, long-day; *D*, warm, long-day.

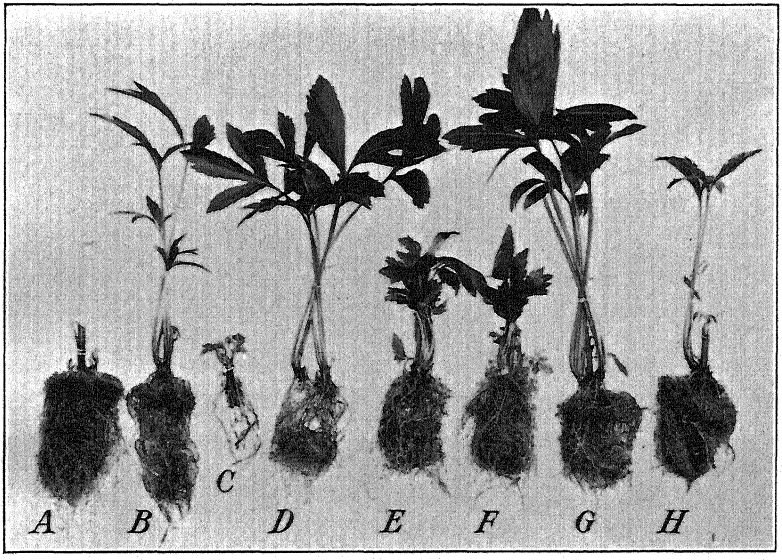


FIGURE 6.—Root production and new foliage regeneration of rudbeckia, photographed April 15: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day (original foliage); *D*, warm, long-day; *E*, warm, long-day to warm, short-day; *F*, cool, short-day to warm, short-day; *G*, cool, long-day to warm, long-day; *H*, warm, long-day to cool, long-day. Note the greater root system of *A* than *B*. *A* is a small plant (fig. 4, *B*, *a*) and *B* is a large plant (fig. 4, *B*, *b*). *B* blossomed earlier than *A*.

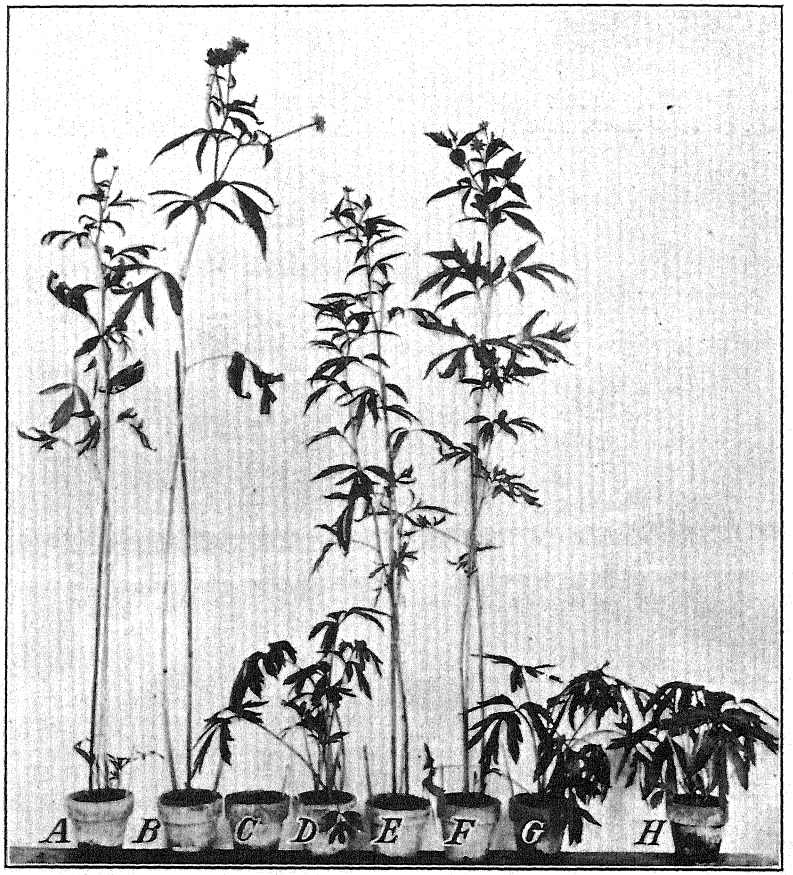


FIGURE 7.—Effect of previous culture upon regeneration of rudbeckia, photographed June 28. Plants under common culture since April 15. Environments of previous culture period as shown in legend of figure 6. The warm, short-day plants died (*C*). Plants with a previous warm, long-day treatment did not produce flower stems.



FIGURE 8.—Klondike cosmos, photographed March 22: *A*, Cool, short-day; *B*, cool, long-day; *C*, medium-temperature, normal-day (the only fruiting plants); *D*, warm, short-day (just beginning to elongate preceding blossoming, since the day was shortened to 9 hours March 1); *E*, warm, long-day.

The horticultural variety of Klondike cosmos, Orange Flare, which blossoms in midsummer, gave a most interesting response. It was assumed that this variant of the short-day Klondike was a long-day type. The results of the environmental treatments proved otherwise. In the cool-temperature environment it did give a short-day response, but in the warm house it blossomed under both the short- and long-day treatments (fig. 10). Very obviously, therefore, this variety blossoms in the summertime out of doors because of high temperatures and not because of long days.

Probably the most interesting plant from a historical viewpoint is Maryland Mammoth tobacco, for it was from the short-day reactions of this variety that the photoperiodism concept originated (*1*). To

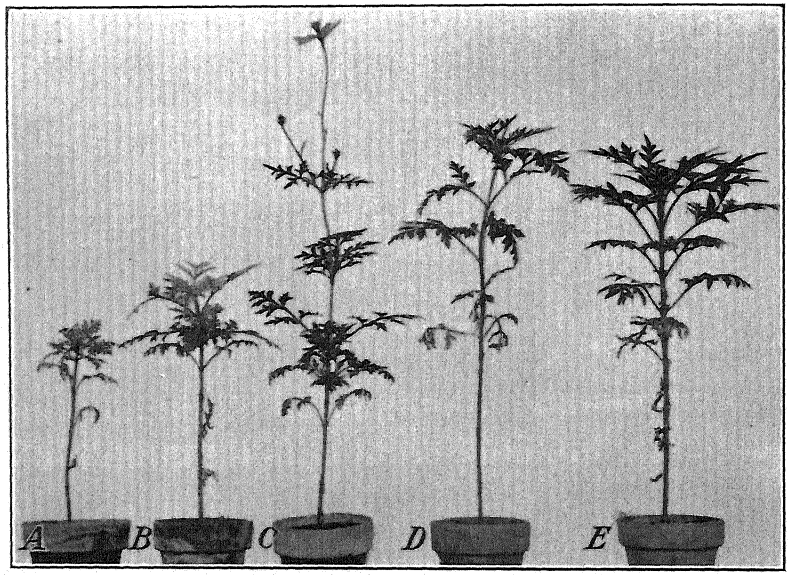


FIGURE 9.—Young plants of Klondike cosmos. Only the intermediate-temperature plants blossomed *A*, Cool, short-day; *B*, cool, long-day; *C*, intermediate-temperature normal-day; *D*, warm, short-day *E*, warm, long-day.

those who would be inclined to look upon photoperiod as having a specific effect in inducing reproduction, in contrast to the view that blossom-bud formation depends upon the internal condition of the plant (*5*), this tobacco would naturally be a key plant by which to test the specific nature of photoperiodism. Seedling plants started in long-day warm conditions in September were still vegetative in May. Plants which were old enough to have several leaves, became reproductive in only 3 or 4 weeks after being transferred to the short-day, warm environment. The rate of growth was slower in the short-day, cool location, but blossom buds were soon formed. The plants in the long-day cool location took on the growth habits of reproductive plants within a few weeks after being transferred, and clearly formed blossom buds after 2½ to 3 months' exposure (fig. 11). That is, Maryland Mammoth tobacco became reproductive in long days when growing at a cool temperature.

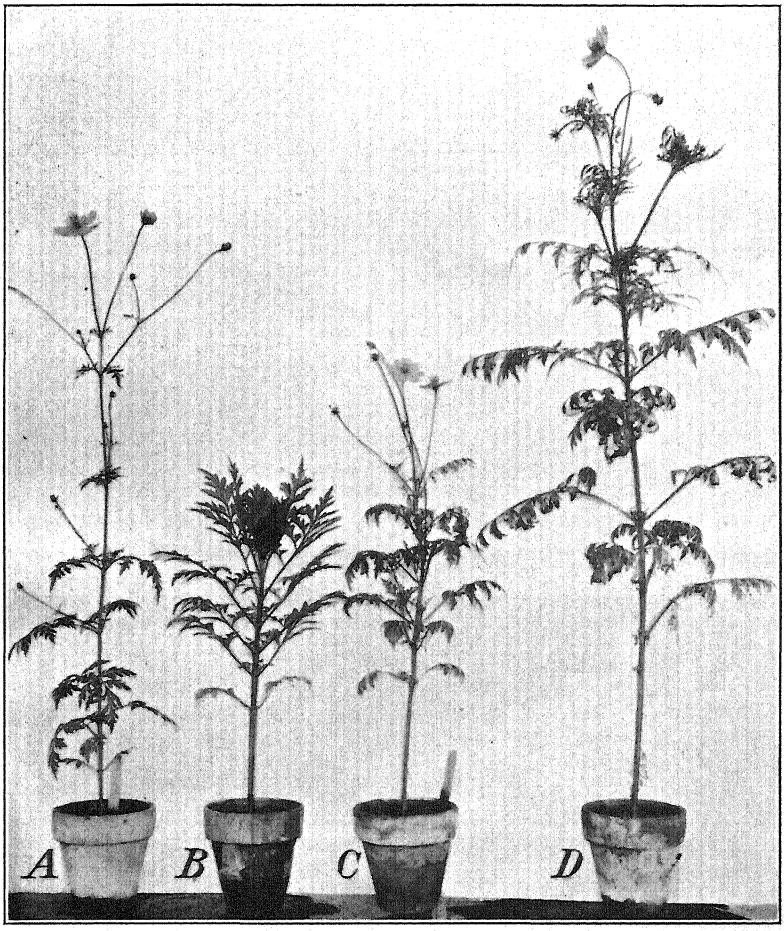


FIGURE 10.—Orange Flare cosmos, a summer-blossoming variant of Klondike: *A*, Cool, short-day; *B*, cool long-day; *C*, warm, short-day; *D*, warm, long-day. The long-day blossoming of this variety illustrates a temperature "sport."

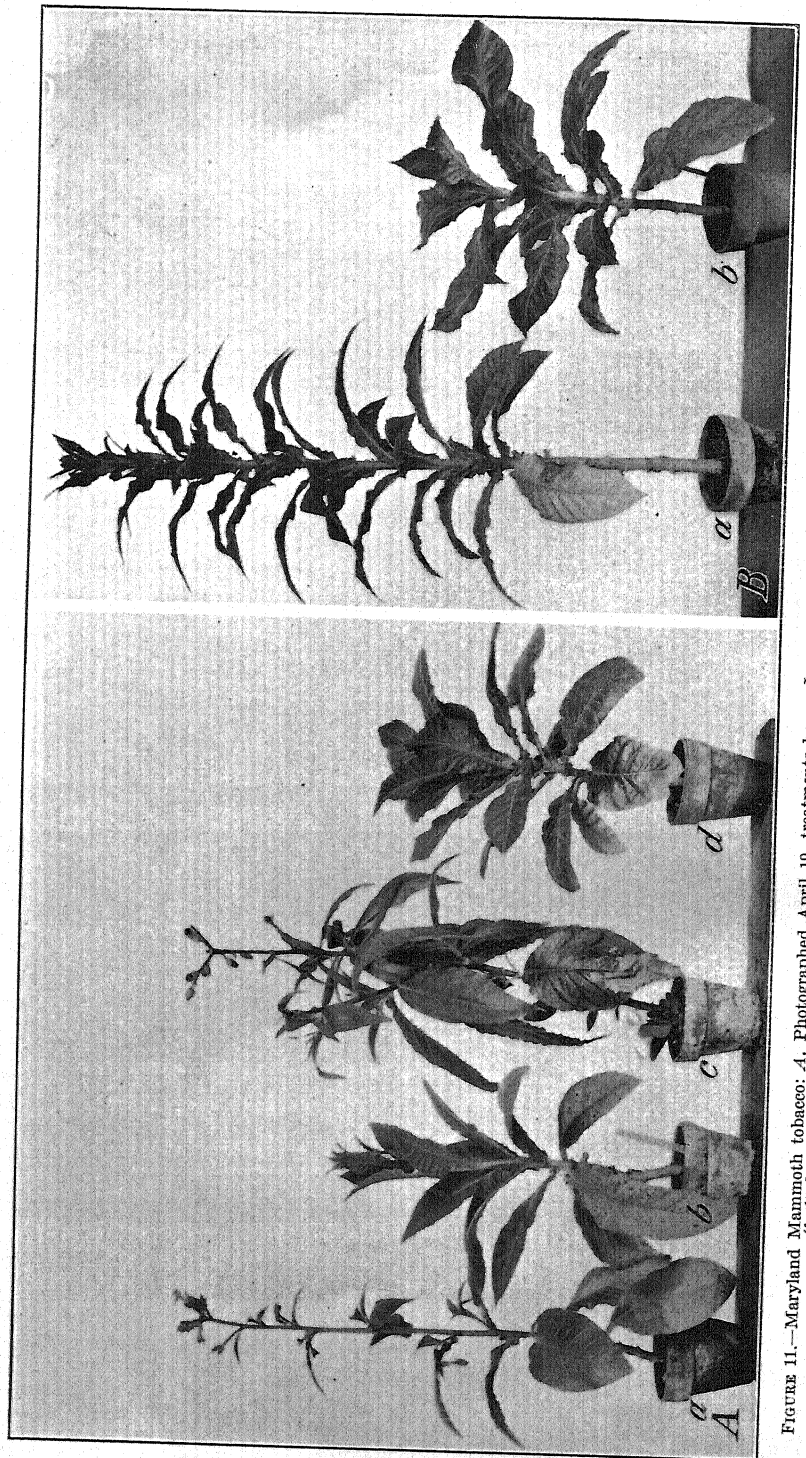


FIGURE 11.—Maryland Mammoth tobacco: *A*, Photographed April 10, treatments begun January 15 *a*, Cool, short-day; *b*, cool, long-day (budding); *c*, warm, short-day (fruited and regenerating;) *d*, warm long-day. *B*, Photographed May 20: *a*, Cool, long-day (budding); *b*, warm, long-day.

Some other responses of particular interest follow: The tobacco variety Havana No. 38 (fig. 12) blossomed first in long days in the warm temperature, but in all environments after different lengths of time. Other plants giving a similar reaction were buckwheat,



FIGURE 12.—Havana No. 38 tobacco blossomed first in long days but eventually in all environments: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day.

fuchsia (fig. 2), mustard (white), nasturtium (fig. 13), New Zealand spinach, and pepper. Morning-glory (var. Heavenly Blue) blossomed in short-day warm and long-day cool locations (fig. 14). When the day length was shortened from the normal winter day of March to 9 hours, cosmos and poinsettia plants which had remained vegetative all winter in the warm house became reproductive.

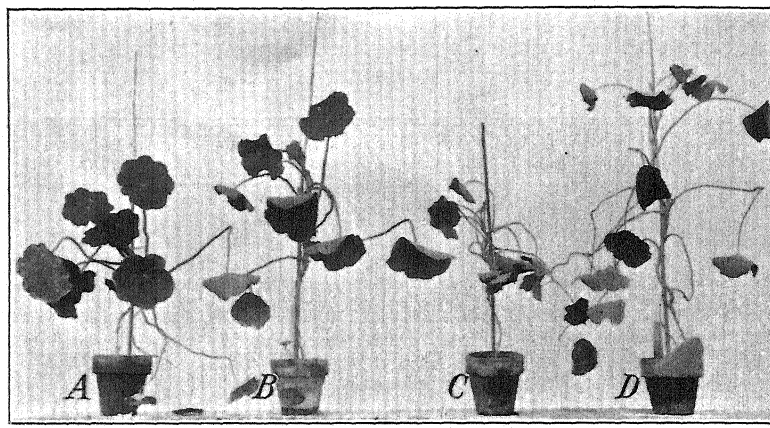


FIGURE 13.—Nasturtium budded and flowered in all environments but after different lengths of time: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day.

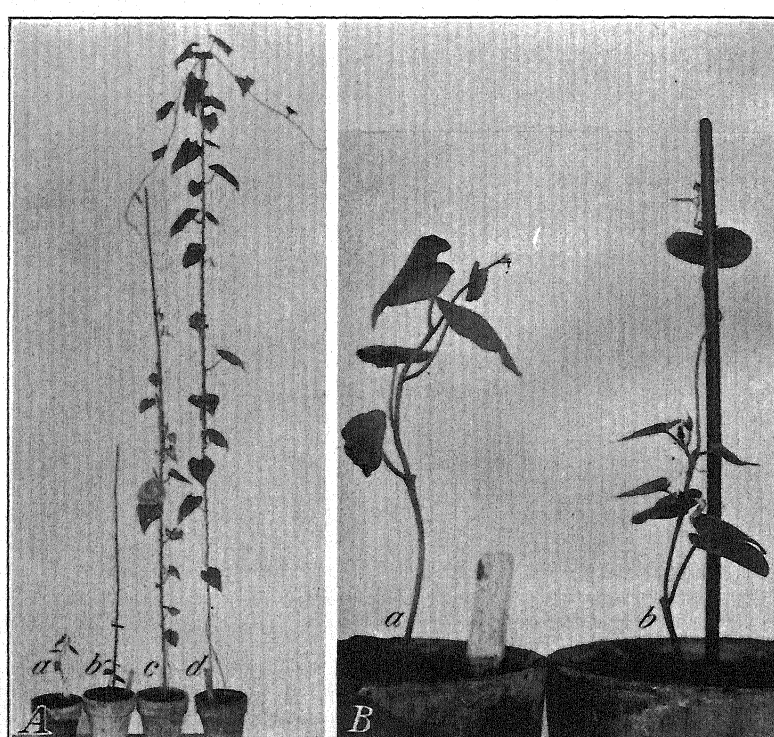


FIGURE 14.—Morning-glory (Heavenly Blue) blossomed in warm, short-day and cool, long-day environments: *A*: *a*, Cool, short-day; *b*, cool, long-day; *c*, warm, short-day; *d*, warm, long-day. *B*: Close-up of cool short-day (*a*) and cool, long-day (*b*) plants.

Some plants blossomed in only one of the environmental combinations, for example, bluegrass (short-day cool) (fig. 15), chrysanthemum var. Lillian Doty (short-day warm) (fig. 16), Cleome (long-day warm) (fig. 17), white clover (long-day cool) (fig. 18), and salvia (short-day cool). Others blossomed in one temperature with relatively little regard to length of day. Examples of these were German stocks (cool) (fig. 19), field corn (warm) (fig. 20), bush bean (warm), pansy (cool), phlox (warm) (fig. 21), and geranium (cool) (fig. 22). A few plants gave similar photoperiodic reactions in both the warm and cool environments. These were spring oats (var. Victory) (fig. 23), winter oats (fig. 24), spring barley (Oderbrucker), chicory (fig. 25), lambsquarters, fall aster, Shasta daisy, dogfennel (fig. 26), annual sugar beet (fig. 27), and possibly *Melilotus dentata* (fig. 28).

No blossoms were produced on such species as potato (figs. 29, 30), sweetpotato, and variegated geranium in any of the environments provided.

Canada thistle, plantain, and wild sunflower plants made no appreciable growth throughout the winter in any of the four environments. Plants of these species collected after the ground had thawed in April grew readily, suggesting that a period of chilling had broken their "rest period."

The species and varieties which gave different photoperiod responses at different temperatures during the winter and spring of 1936–37 were: Alfalfa (seed setting), winter barley, castor-beans, bush beans, beets (fig. 31), bulbous bluegrass, Kentucky bluegrass, bryophyllum (fig. 32), Chinese cabbage, reed canary grass (fig. 33), celery, chrysanthemum var. Lillian Doty, Cleome, white clover, corn var. Golden Glow, Klondike cosmos, Orange Flare cosmos, cucumber, geranium, gourd, hemp, jimsonweed (fig. 34), lettuce (fig. 35), morning-glory, pansy, pigweed, phlox, plumbago, poinsettia, quackgrass (fig. 36), rudbeckia, spring rye, winter rye, salvia, soybean var. Illini (fig. 37), squash, stock, Sudan grass, sowthistle, timothy (figs. 38, 39), Maryland Mammoth tobacco, tumbleweed (fig. 40), spring wheat, and winter wheat (fig. 41).

The unlike responses of different branches or parts of a plant when these are given different photoperiod environments constitute one of the interesting angles of the phenomenon of photoperiodism (*2*, *6*). This customary result was not secured when morning-glory plants were placed with part of the plant exposed to one photoperiod treatment and the remainder to another. At warm temperature the variety Heavenly Blue behaved as a short-day plant. Plants with the bases (2 feet) and lower branches in a short-day environment and the tops in a long-day blossomed throughout. Plants with the bases in a long-day environment and the tops in a short-day gave a delayed response but also blossomed in both environments. Plants in a long-day environment did not blossom (fig. 42). Unlike some other species, the morning-glory appears to respond systemically and not locally. Also the reproductive state may originate in the base as well as in the upper portion.

In the case of plants which were unfavorably affected in their growth and fruiting responses by the warm environment, added light (long-day treatment) frequently aggravated the temperature injury instead of being a compensating factor (figs. 15, 31, 33).

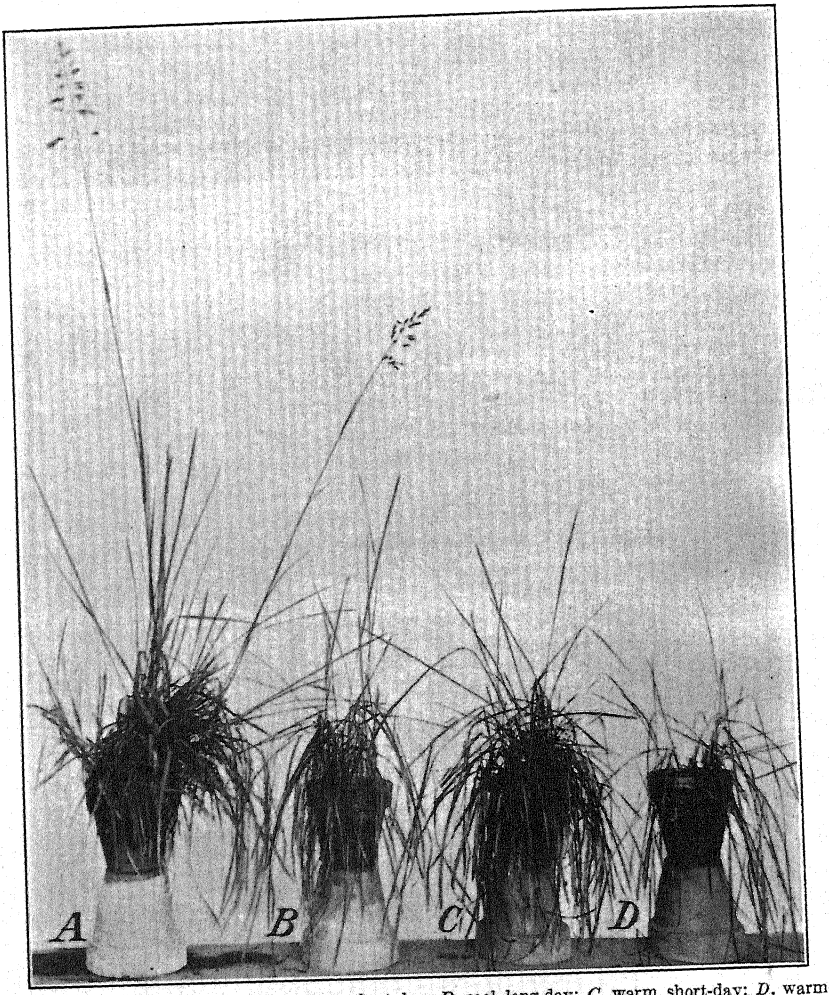


FIGURE 15.—Kentucky bluegrass: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day.

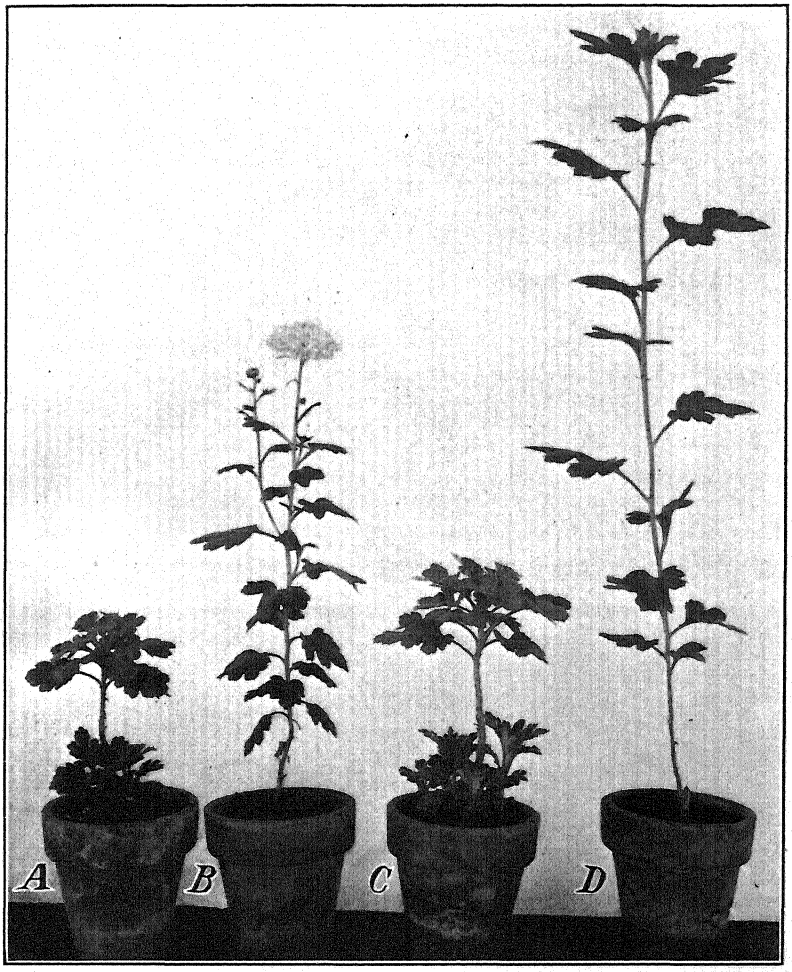


FIGURE 16.—Chrysanthemum var. Lillian Doty: *A*, Cool, short-day; *B*, warm, short-day; *C*, cool, long-day *D*, warm, long-day. Plants that are kept cool sucker instead of producing stems. These plants were practically the size shown when the treatments began.



FIGURE 17.—Cleome thrives only in long days and at warm temperature: *A*, Cool, short-day; *B*, cool long-day; *C*, warm, short-day; *D*, warm, long-day.

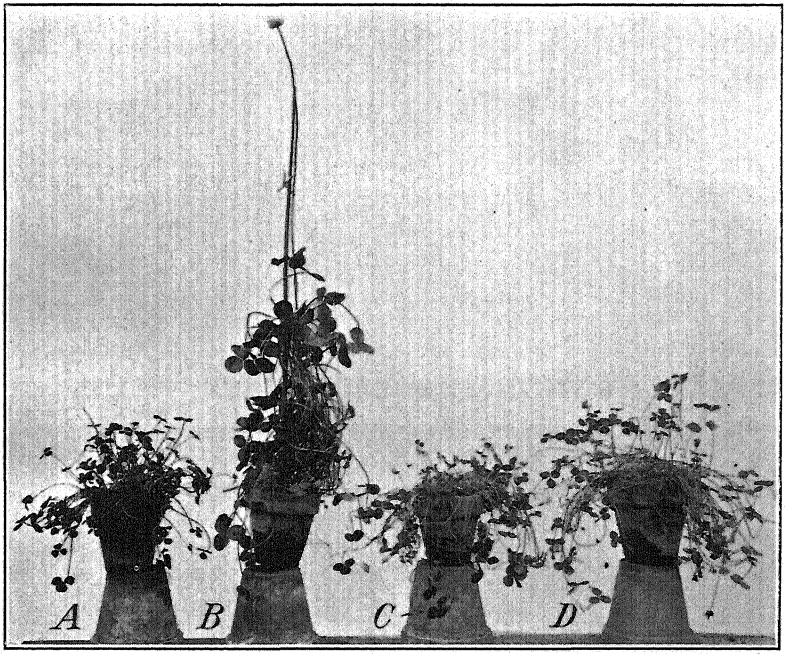


FIGURE 18.—White clover becomes a "giant" plant in a cool, long-day environment: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day.

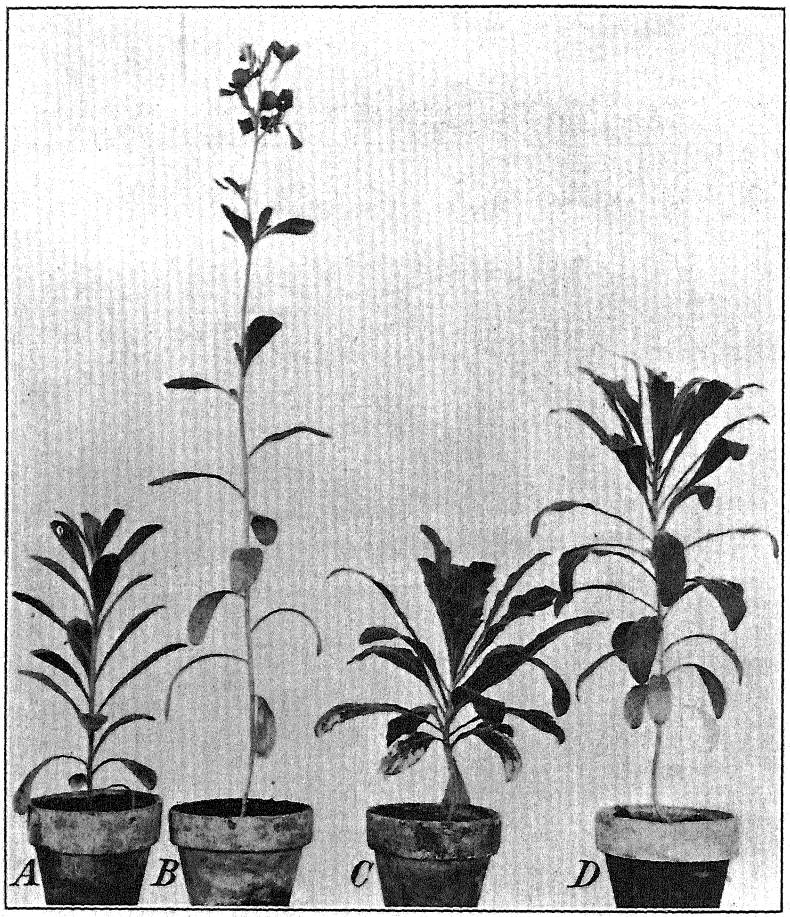


FIGURE 19.—Stocks blossom only at cool temperatures: *A*, Cool, short-day (budding); *B*, cool, long-day *C*, warm, short-day; *D*, warm, long-day.

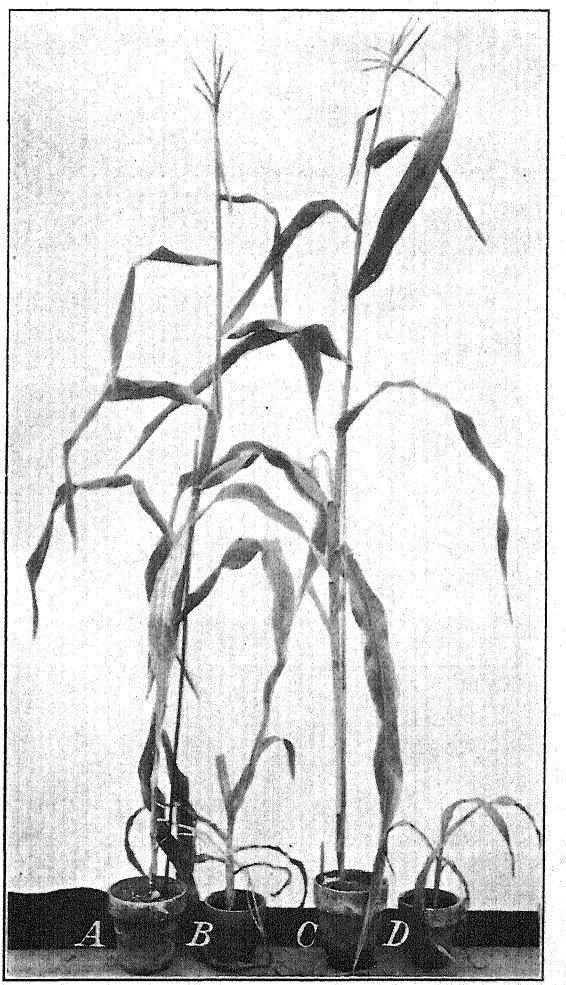


FIGURE 20.—Corn (Golden Glow) is insensitive to photoperiod at high temperatures, although it responds at intermediate temperatures (*6*): *A*, Warm, short-day; *B*, cool, short-day; *C*, warm, long-day; *D*, cool, long-day.

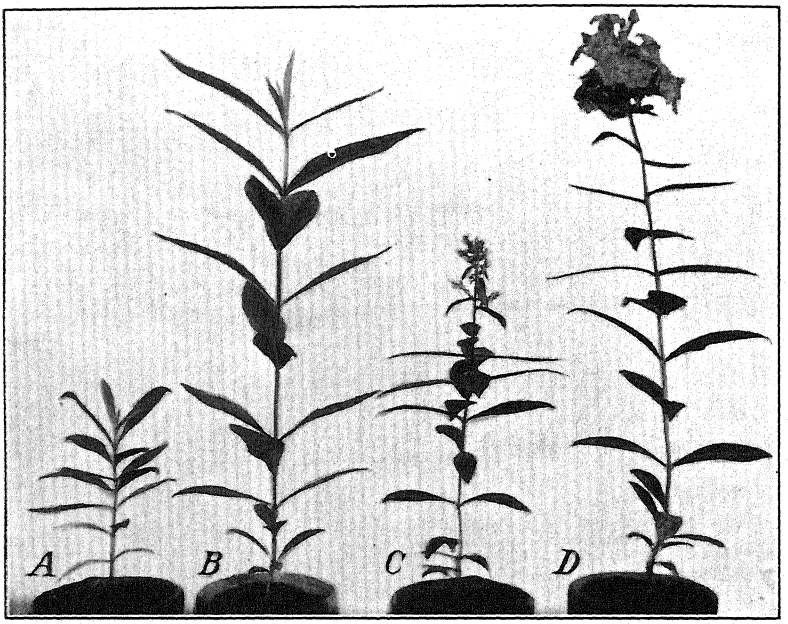


FIGURE 21.—Phlox blossoms at warm temperatures: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day.

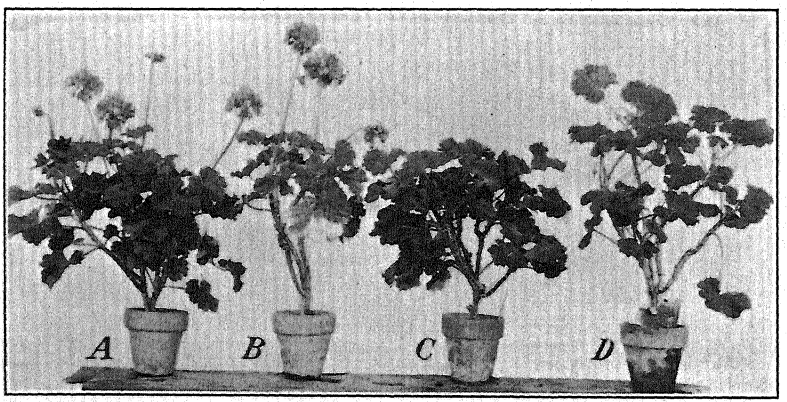


FIGURE 22.—Geranium blossomed better in a cool temperature: *A*, Cool, short-day; *B*, cool, long-day *C*, warm, short-day; *D*, warm, long-day.

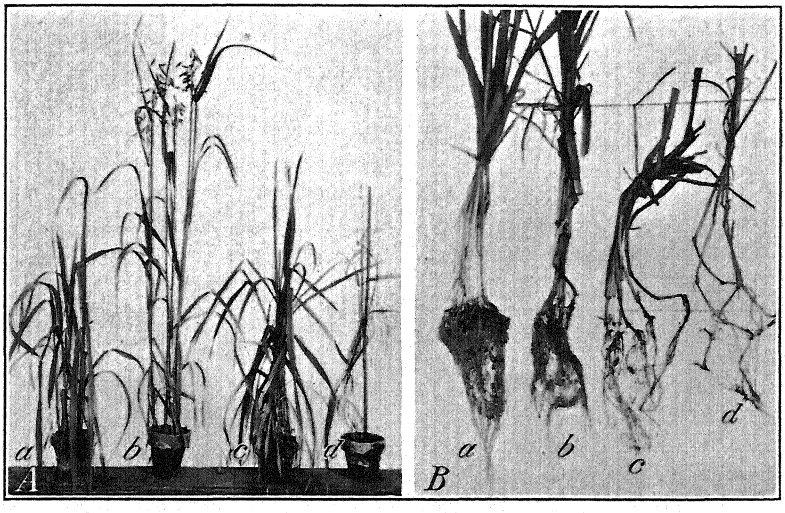


FIGURE 23.—Victory oats: *A:* *a*, Cool, short-day; *b*, cool, long-day; *c*, warm, short-day; *d*, warm, long-day. *B:* Roots of plants in *A*. Fruiting plants had less roots; early fruiting (*d*) had much less than if a good leaf development had preceded heading (*b*). Compare with figure 41.

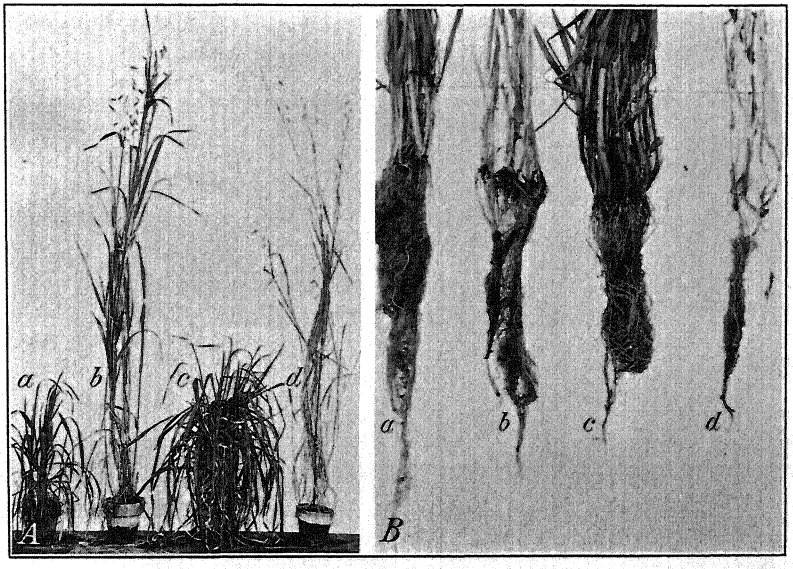


FIGURE 24.—Winter oats shows more adaptation to temperature than Victory (fig. 23). With more foliage it also shows more rooting; same arrangement of plants and lettering as in figure 23.

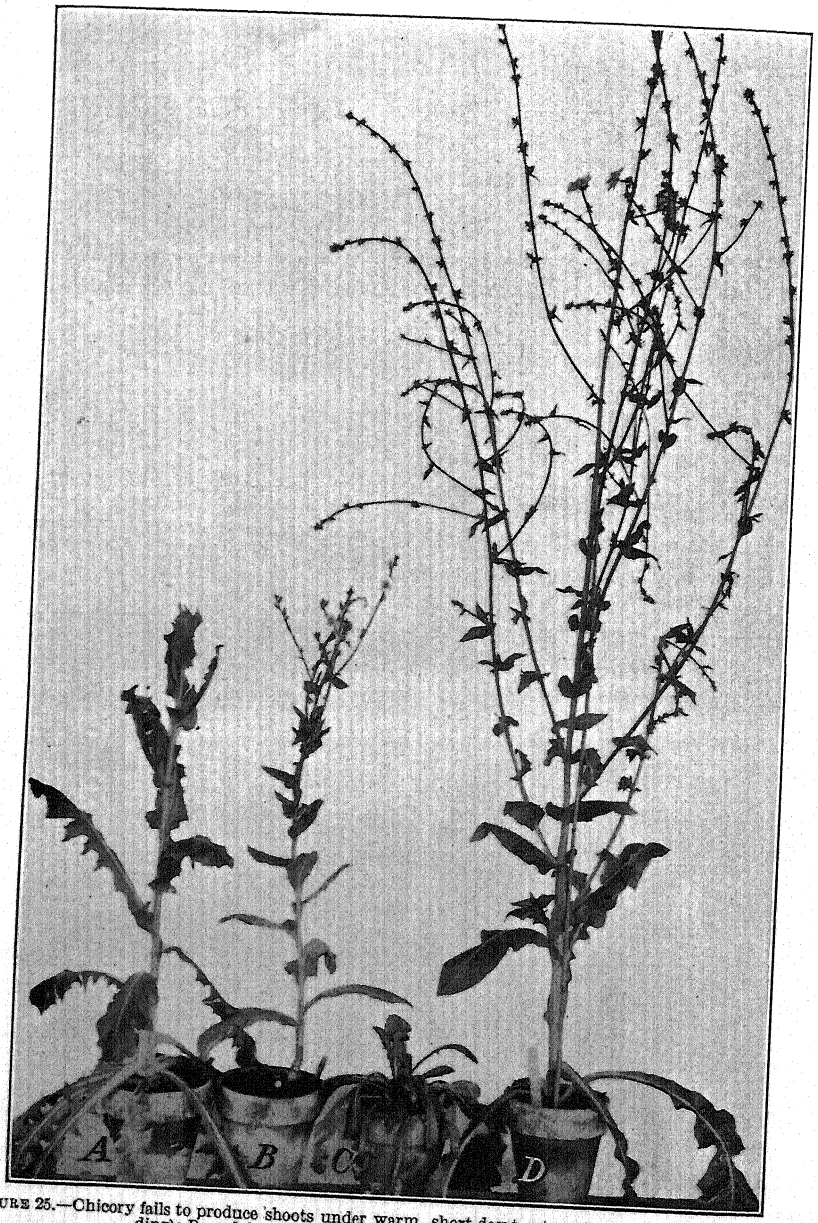


FIGURE 25.—Chicory fails to produce shoots under warm, short-day treatment: *A*, Cool, short-day (budding); *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day.

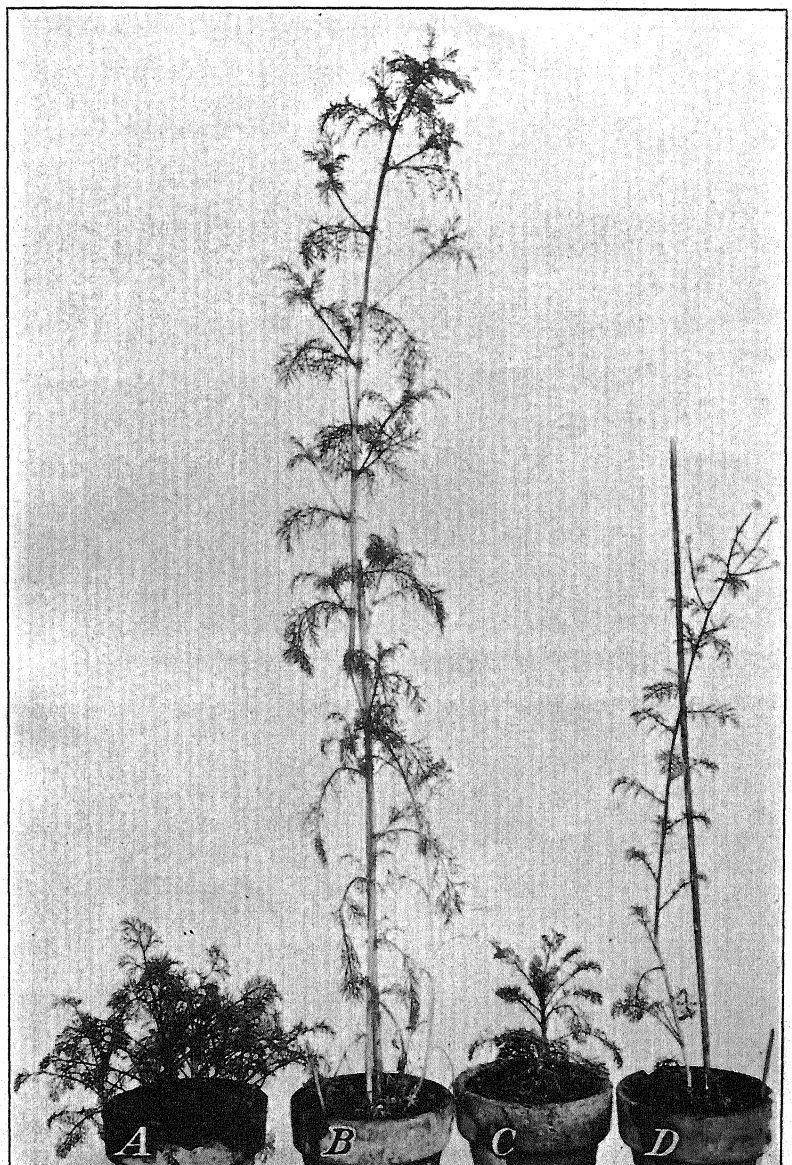


FIGURE 26.—Dogfennel blossoms about equally at different temperatures: *A*, Cool, short-day; *B*, cool long-day; *C*, warm, short-day; *D*, warm, long-day.



FIGURE 27.—Annual sugar beets (No. 2240) need long days to produce first-season "bolting": *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day. Compare with figure 31.

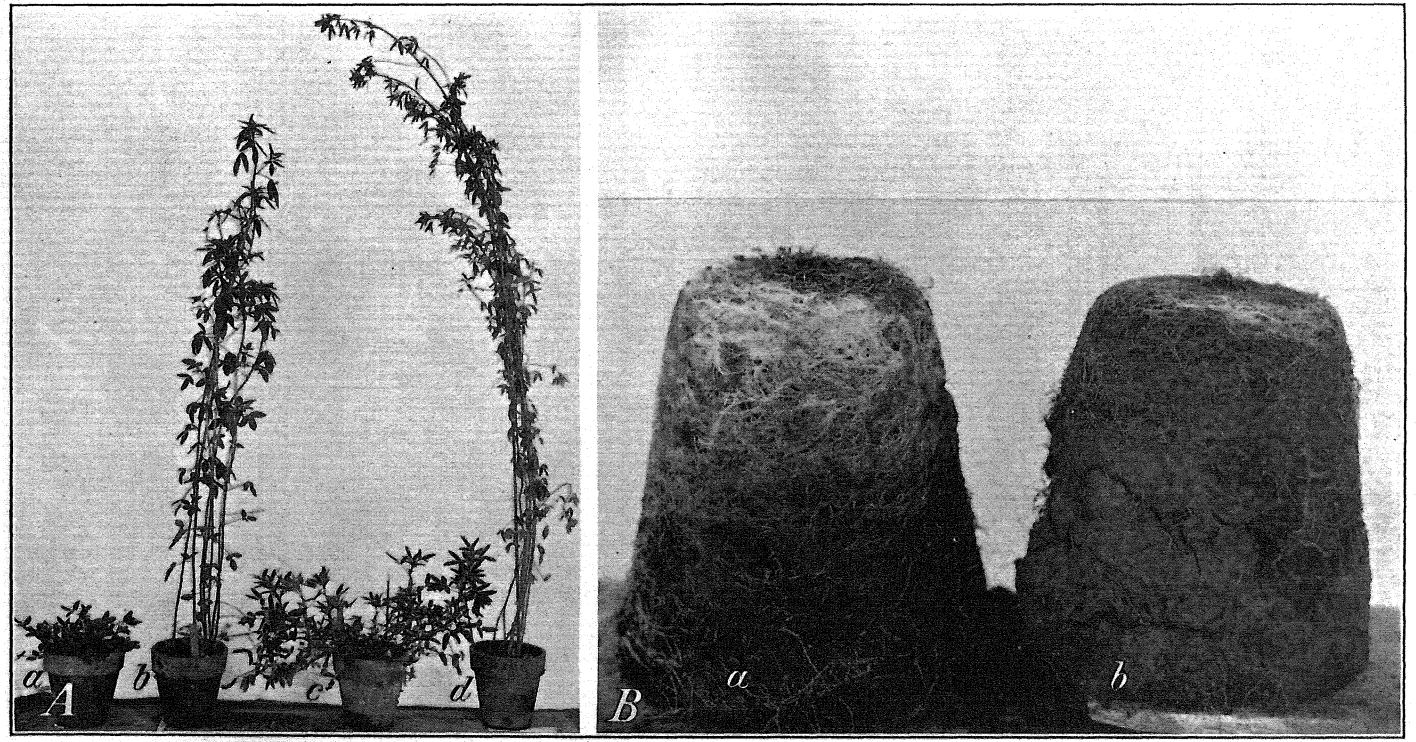


FIGURE 28.—Siberian sweetclover (*Melilotus dentata*), 8-weeks treatment: *A:* *a*, Cool, short-day; (*b*), cool, long-day (budding); *c*, warm, short-day; *d*, warm, long-day (blossoming). *B:* Soil from pots of warm, short-day (*a*) and warm, long-day (*b*) plants showing root differences. The tall, fruitful plant has fewer roots.

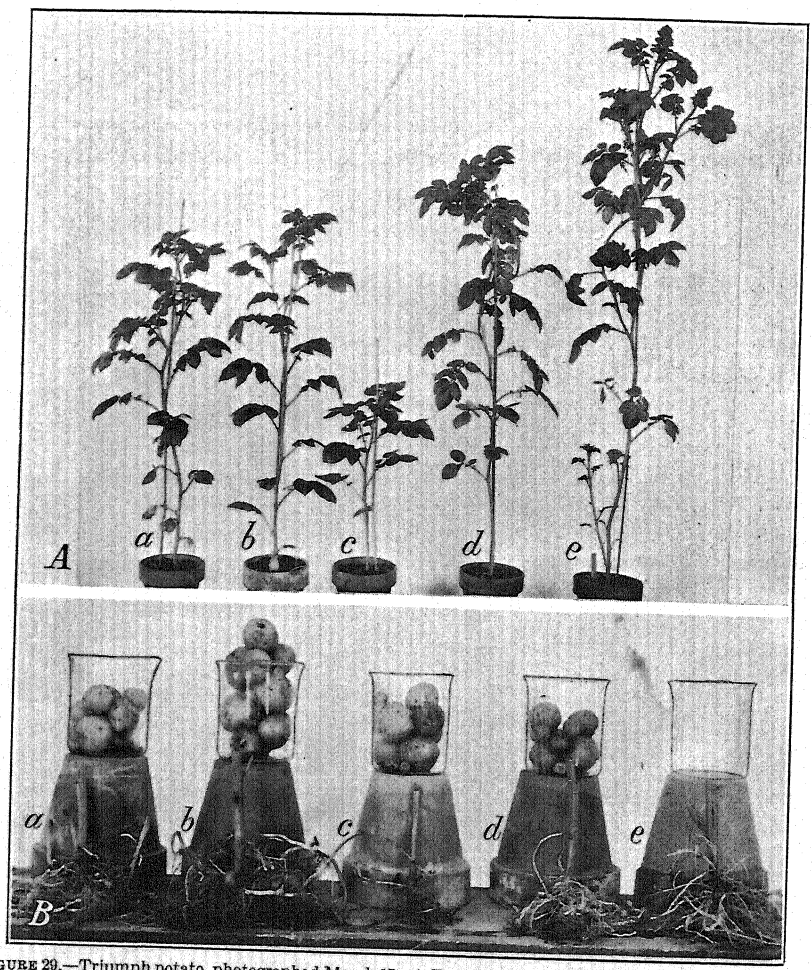


FIGURE 29.—Triumph potato, photographed March 17: *A*, Top growth: *a*, Cool, short-day; *b*, cool, long-day; *c*, medium temperature, normal-day; *d*, warm, short-day; *e*, warm, long-day. *B*, Roots of one plant and tubers from three plants of each lot arranged and lettered as in *A*.

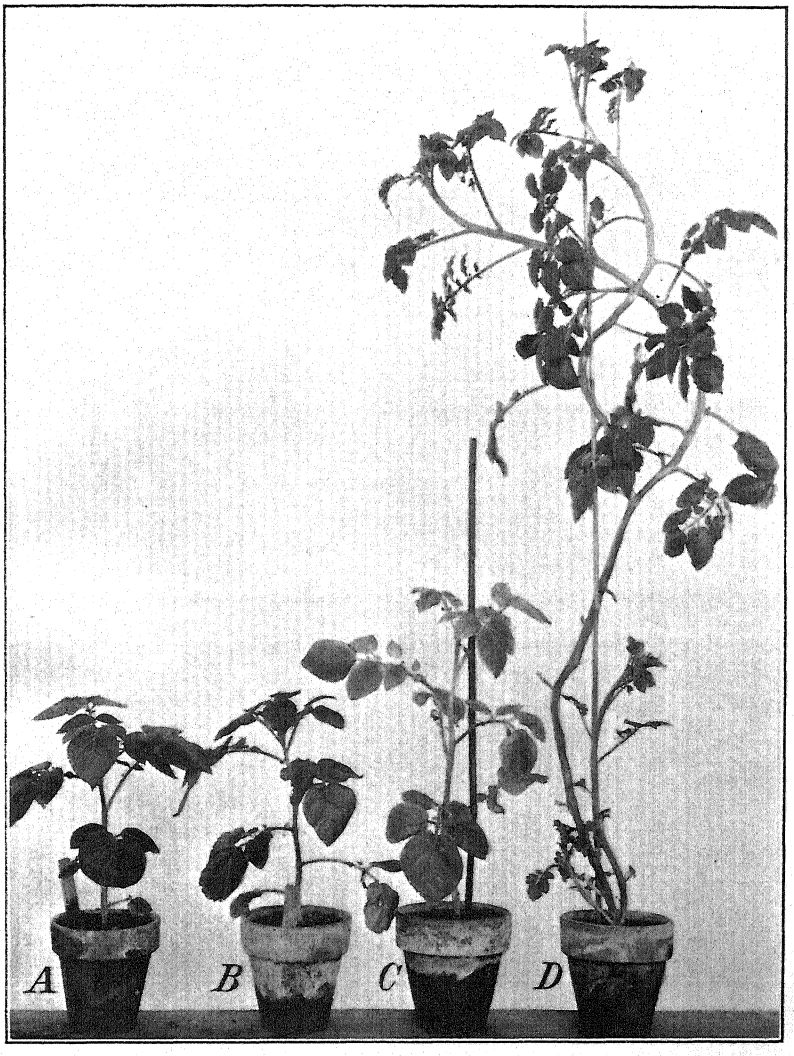


FIGURE 30.—Irish Cobbler potato grows slowly except under long-day, warm condition (compare with fig. 29, *A*): *A*, Cool, short-day; *B*, cool, warm-day; *C*, warm, short-day; *D*, warm, long-day.



FIGURE 31.—Table beet var. Early Wonder; *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day. Compare with figure 27.

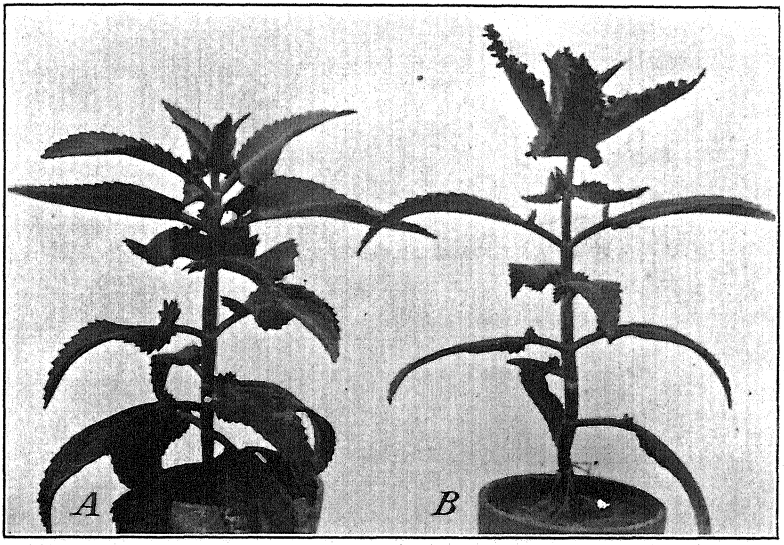


FIGURE 32.—Long-day (*B*) bryophyllum plants regenerate abundantly; short-day plants do not (*A*).



FIGURE 33.—Reed canary grass: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day; *D*, warm, long-day.

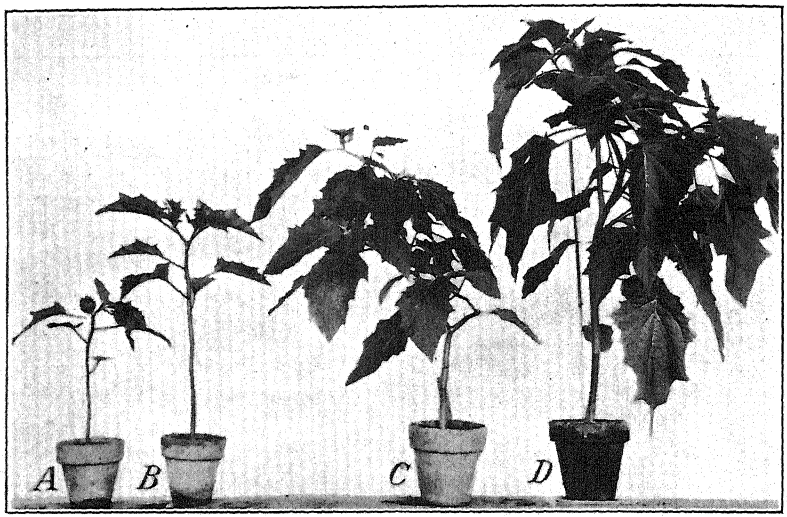


FIGURE 34.—Jimsonweed responded to temperature as well as to photoperiod: *A*, Cool, short-day (fruiting); *B*, cool, long-day (blossoming); *C*, warm, short-day (blossoming); *D*, warm, long-day (vegetative).

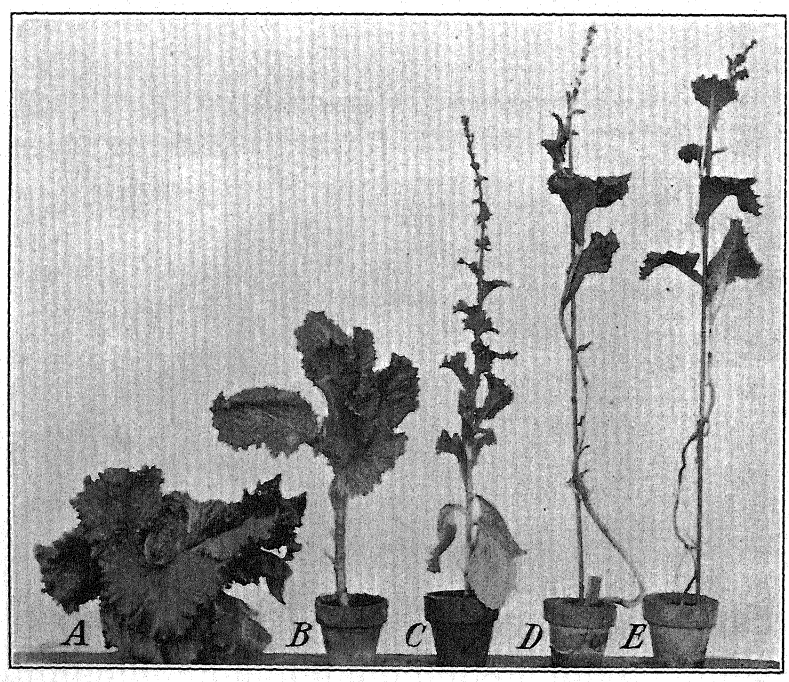


FIGURE 35.—Lettuce blossoming varied with temperature, nutrient, and photoperiod: *A*, Cool, short-day; *B*, cool, long-day (beginning to bolt); *C*, cool, long-day (same age as *B*) in sand culture (low nitrogen); *D*, warm, short-day; *E*, warm, long-day.

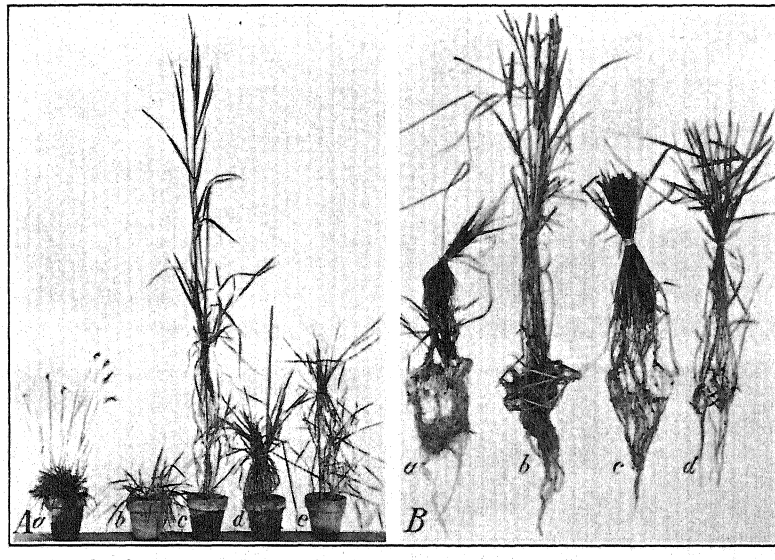


FIGURE 36.—Quackgrass: *A*: *a*, "Fruiting" habit of *Poa bulbosa*, a cool, short-day-preference plant; *b*, cool, short-day; *c*, cool, long-day (heading); *d*, warm, short-day; *e*, warm, long-day (sterile stems). *B*: Roots of quackgrass plants arranged as in *A*. Fruiting plants (*b*) are producing underground shoots.

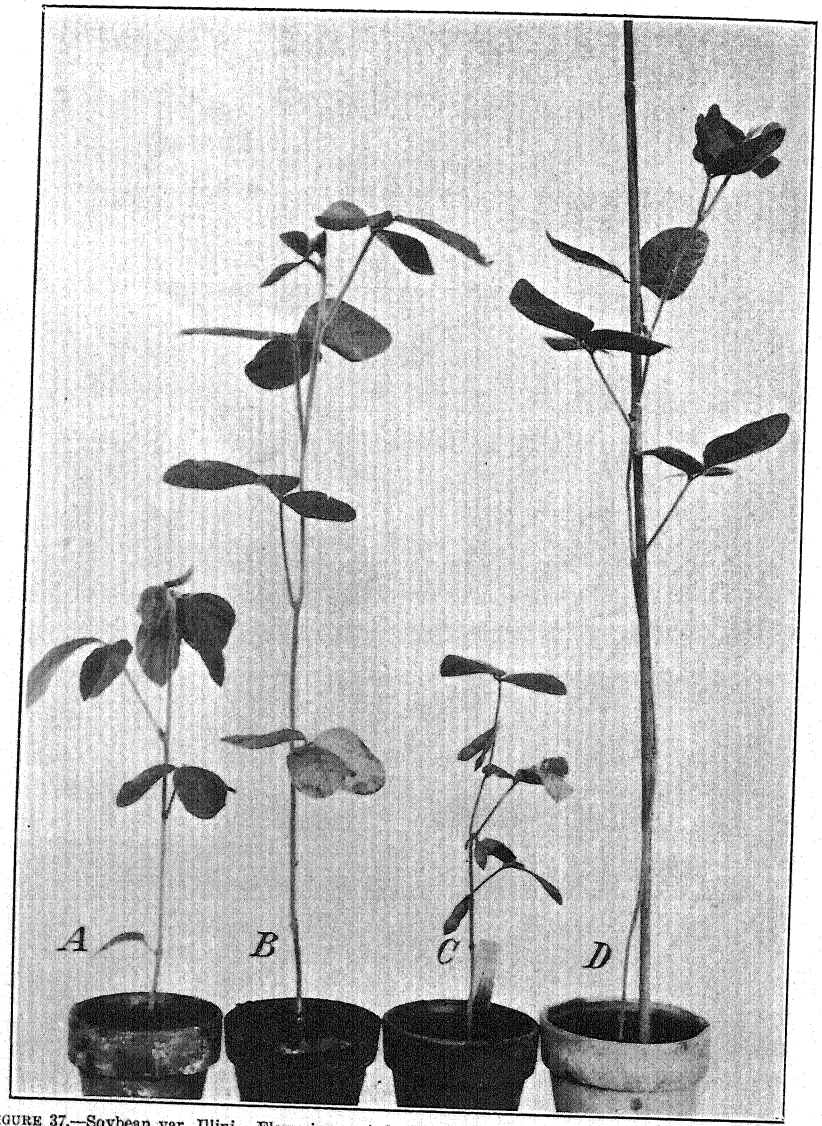


FIGURE 37.—Soybean var. Illini. Flowering varied with temperature as well as photoperiod: *A*, Cool, short-day; *B*, cool, long-day; *C*, warm, short-day (fruiting); *D*, warm, long-day.

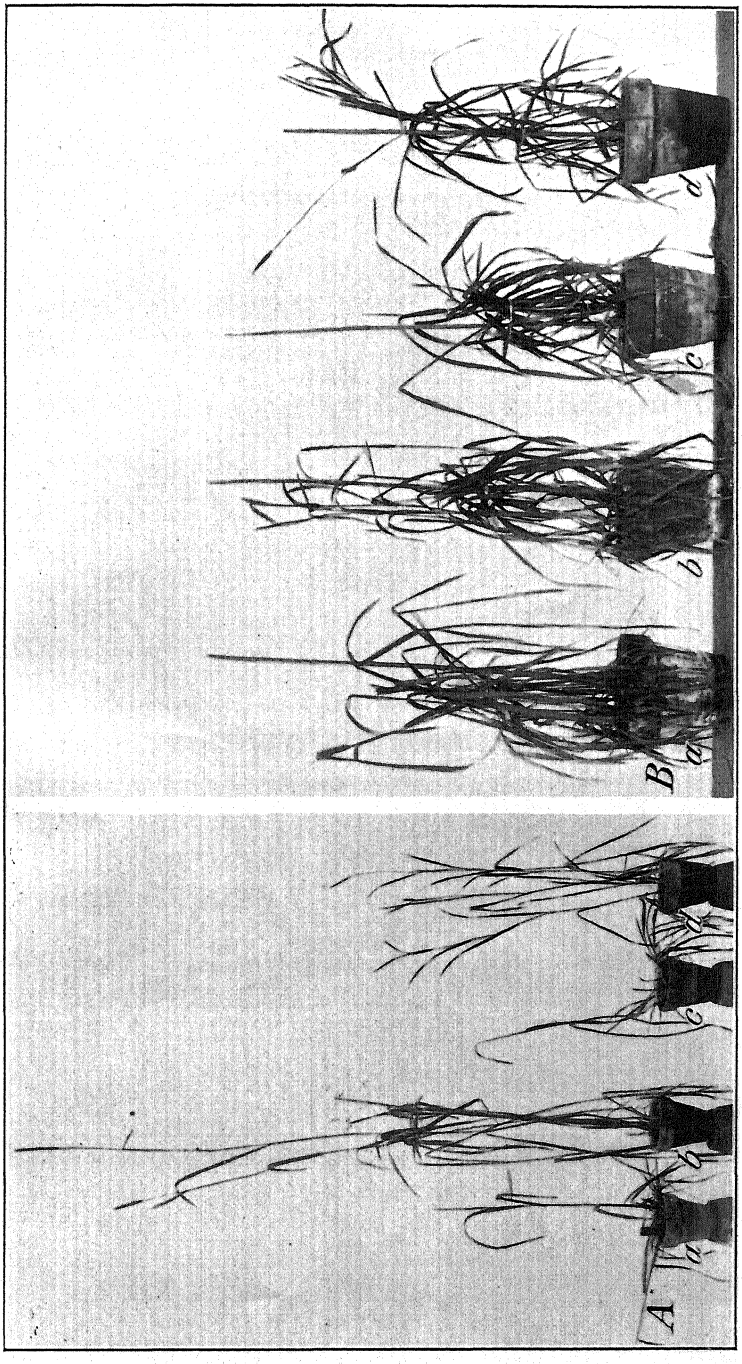


FIGURE 38.—Two unknown varieties of timothy (*A* and *B*), showing different responses to environment: *a*, Cool, short-day; *b*, cool, long-day; *c*, warm, short-day; *d*, warm, long-day.

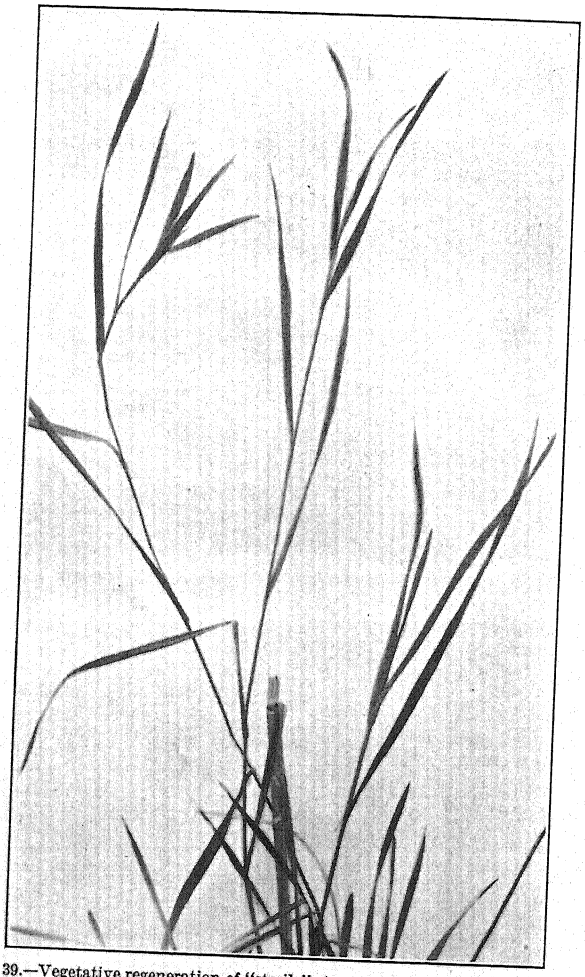


FIGURE 39.—Vegetative regeneration of "sterile" stems of timothy. See figure 38, *A*, *d*.

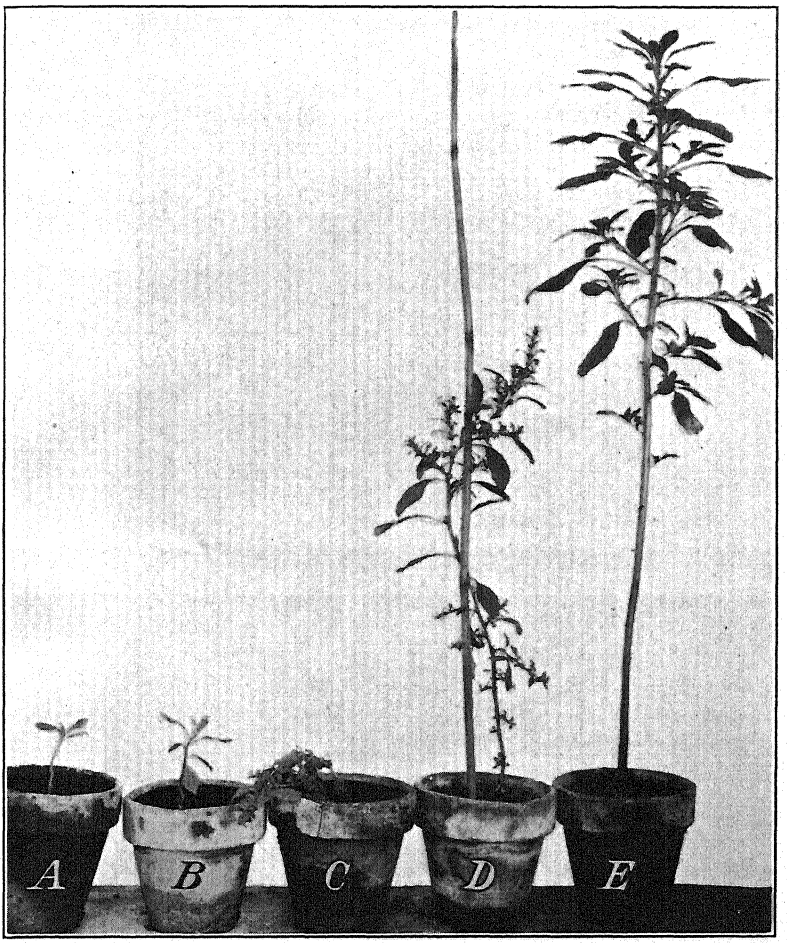


FIGURE 40.—Tumbleweed, photographed March 27: *A*, Cool, short-day; *B*, cool, long-day; *C*, medium-temperature, normal-day (fruiting); *D*, warm, short-day (fruiting); *E*, warm, long-day (vegetative).

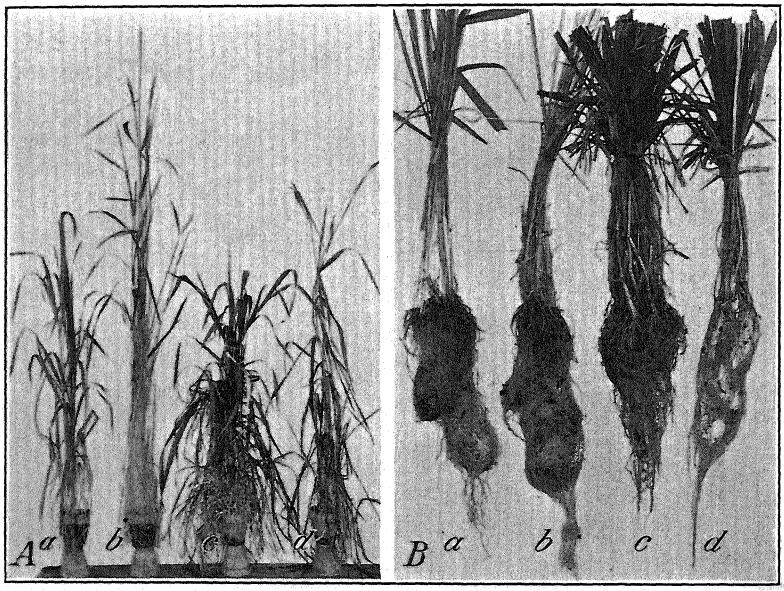


FIGURE 41.—Winter wheat: *A*, Tops: *a*, cool, short-day; *b*, cool, long-day; *c*, warm, short-day; *d*, warm, long-day. *B*, Abundant roots follow leafy tops. Lettering in same order as *A*. Compare with figures 23 and 24.

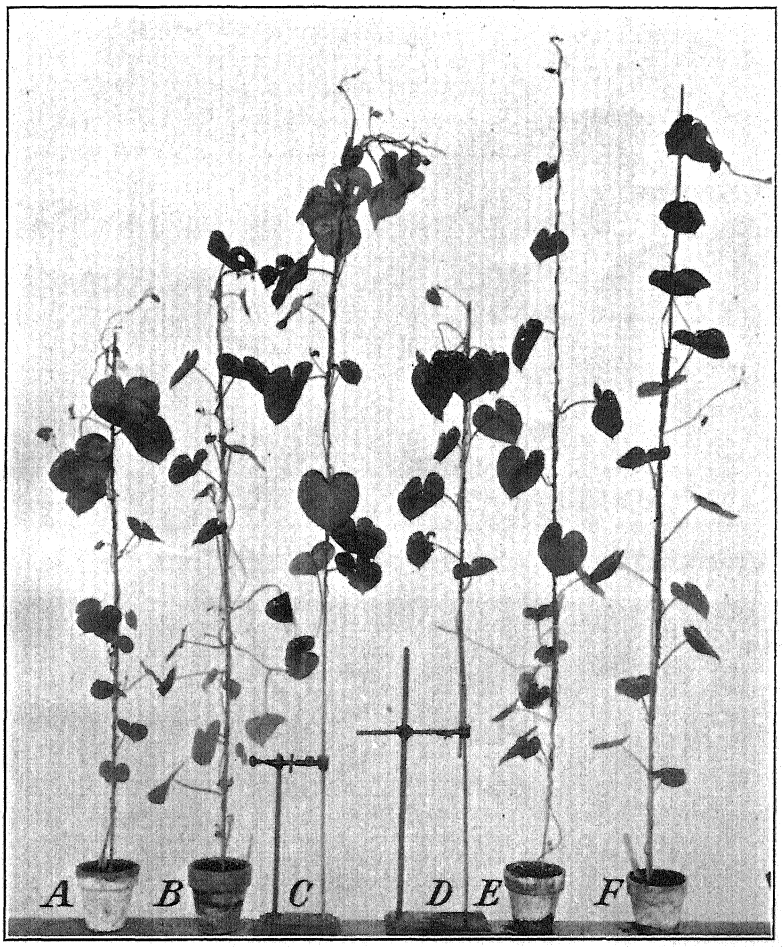


FIGURE 42.—Plants of warm-temperature morning-glory var. Heavenly Blue: *A*, Short-day (blossoming); *B*, base and branches, short-day (blossoming); *C*, top of plant *B*, long-day (blossoming); *D*, top of plant *E* short-day (budding); *E*, base and branches, long-day (budding); *F*, long-day (vegetative). Blossoming was induced by any part of the plant being under short-day treatment.

From the performance of some plants such as portulaca, dianthus, and celery (fig. 46) it appears that variable temperatures and/or light conditions would be more conducive to flowering than uniform environmental conditions.

OTHER ENVIRONMENTAL FACTORS

Other treatments than temperature were used with a few varieties of plants to affect the initiation of the reproductive state. Like temperature and photoperiod, a low nitrogen nutrient (bank-sand culture) may have opposite effects upon the time of blossoming of different plants. For example, this nutrient condition hastened the flowering of *Clarkia* but delayed it in the case of marigold (fig. 43). Previously

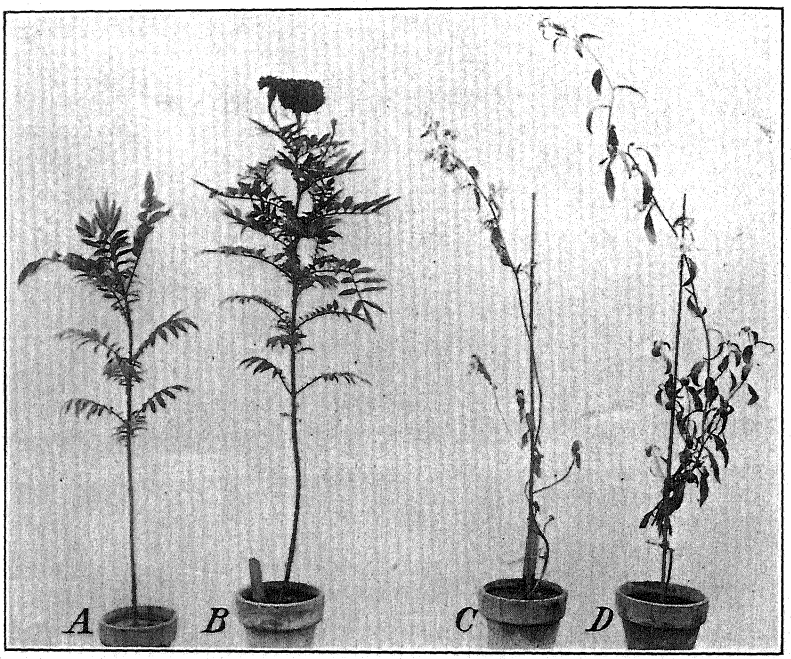


FIGURE 43.—Nutrient affects blossoming of different plants unequally: *A*, Marigold in sand (vegetative); *B*, same in soil (blossoming); *C*, Clarkia in sand (blossoming); *D*, same in soil (budding).

blossoming geranium plants which were made vegetative by a warm, short-day treatment blossomed in this environment when transferred from soil to sand.

Girdling did not induce flowering of Klondike cosmos or poinsettia. This technique is noted for giving variable results, being effective in those situations where the girdled plant is "close to becoming reproductive" (fig. 44). For instance, varieties of apples which are "early bearers" will usually make a striking response to girdling, whereas those which are known to be late bearing may show no blossom-bud formation the first season after being girdled.[3]

[3] Unpublished data.

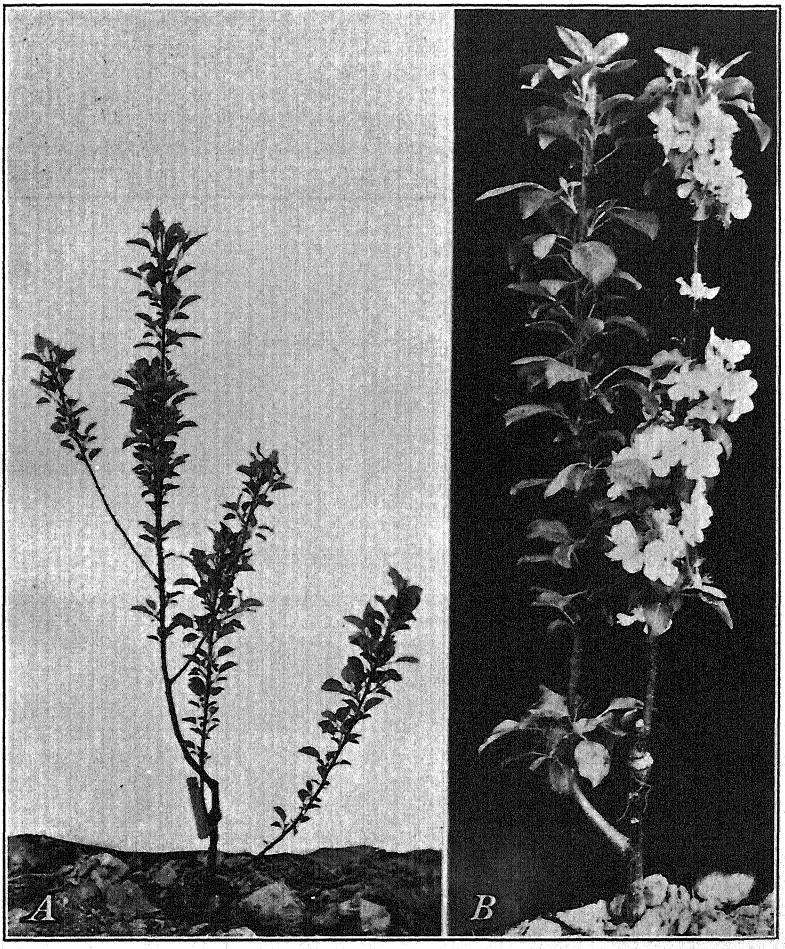


FIGURE 44.—Girdled apple trees: *A*, Winesap, still vegetative; *B*, Wealthy, blossoming. Top and suckers of Winesap much alike in foliage appearanec and bark color; Wealthy top (girdled) unlike sucker in growth characters.

Partial defoliation (fig. 1) prevents or delays blossoming (fig. 45), the degree of influence apparently being in proportion to the readiness with which the plants flower. Plants which produce blossoms at only a few of their growing points are readily inhibited from blossoming by a reduction in leaf area. On the other hand, if it is the habit of the plant to flower abundantly, defoliation has less effect upon blossom production.

Shading in which the light intensity was reduced to 10 to 15 foot-candles, had much the same type of effect as partial defoliation. It should be borne in mind, however, that the effects are different when the plants have a good soil medium than when they are grown in bank-sand culture. In the case of a low nitrogen supply, flowering may be benefited, of course, by partial shading or defoliation.

The attempt to induce bolting of celery by cool temperature was not very successful, as happened in a previous case (*6*). The following interesting result was secured with celery, however, and may explain

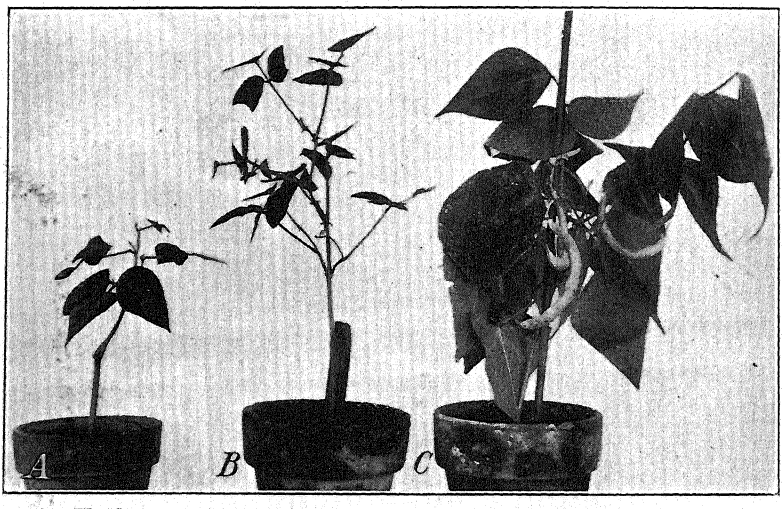


FIGURE 45.—Wax beans: *A*, Cool (vegetative); *B*, warm "defoliated" (vegetative); *C*, warm (fruiting).

the previous failures. While plants started in October did not produce seedstalks by May in any of the four environments, plants in the cool house but in the normal day became reproductive (fig. 46). Obviously, the shifting day length with the advent of spring had a different effect than a continuous long-day treatment. The short-day plants which had been in the cool temperature treatment began to bolt in the warm weather of June. This shows a reaction to a varying length of day at uniform temperature as well as to varying temperature with a uniform length of day.

ROOT DEVELOPMENT

The question of the root development of plants grown in different environments belongs, properly, to a later paper in which the anatomical condition of roots is to be recorded, but some of the striking relations of top and root development that have been observed should be reported without this delay.

The amount of roots the plants produce is obviously related to the extent and character of the foliage development (figs. 23, 24, 28, 29, 36, 41). The flowering state seemed to reduce root formation. Since many plants had a small amount of foliage when flowering began, these also had smaller root systems. If abundant foliage was produced prior to the advent of blossoms the fruiting plants had a more extensive root development. Root extension appears to be related to the external environment only as the latter is reflected in the character of top produced.

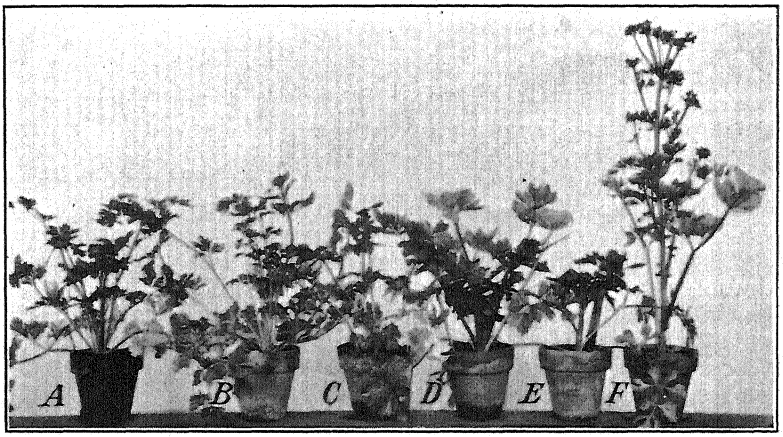


FIGURE 46.—"Bolting" of celery occurred under cool, natural-day rather than under short- or long-day treatment: *A*, Warm, long-day; *B*, warm, short-day; *C*, warm, normal-day; *D*, cool, long-day; *E*, cool, short-day (seedstalks beginning); *F*, cool, normal-day.

DISCUSSION

The view to be taken of the role of photoperiodism in inducing blossoming becomes altered from what it has generally been for the last 17 years when the effects of temperature upon photoperiodic responses are considered. Photoperiod is thus no longer a factor having a specific effect which cannot be much modified, but becomes, along with other external environments, one of the contributing conditions which, taken together, create an internal condition of the plant that results in blossom-bud formation (*5*). For many plants, a certain photoperiod may be a dominant requirement, but it now appears that it is not an essential one. This fact, together with the finding that the carbon dioxide exchange rhythm is correlated with the flowering state (*6*) and that the anatomical conditions of plants appear to affect blossoming (*8*, *12*), is taken as evidence that the production of blossoms may be due to a like physiological condition in many plants and not the result of a different one for each variety. While different external environments are required to induce blossoming of different varieties, the present data seem to indicate that this reaction was the result of a common type of condition within the plant.

Another item of theoretical importance is the reaction of morning-glory when growing with parts of the plant in different environments. Its systemic instead of local response would lead to the suggestion that the deduction, which has been made from some plant habits,

that photoperiod phenomena originate in the apex of the plant, should not become a generalization.

It would now appear probable that much of the uncertainty which is becoming apparent in the literature as to the proper photoperiod classification of some plants has arisen from variations in the cultural treatments other than photoperiod, such as temperature.

The influence of environment upon plant form is very apparent in many series (figs. 18, 19, 26, 28, 33, 40).

The very apparent fact that some varieties within a species show a marked adaptation to widely different environments raises this question: Is the degree of adaptation a genetic character? If it should be more definitely determined after further trials that the ability of a plant to thrive in other than a narrow range of environments is hereditary, this character should be recognized and given special consideration in the breeding and selection of agricultural varieties (figs. 23, 24, 29, 30, 38).

Since a given environment, such as photoperiod or temperature, does not have a specific effect in inducing blossoming but has a varying effect depending upon other factors, in future studies to determine why plants blossom it would seem advisable to give special attention to the internal conditions that influence blossoming. While different factors of the environment may appear at times to have a regulating effect upon the production of blossoms, it would seem that this is so only because those factors induce certain conditions within the plant which result in blossoming.

SUMMARY

The photoperiod responses of a number of plants were altered by temperatures a little above or below those usually employed in greenhouse culture. Among the plants so affected were poinsettia, Klondike cosmos, rudbeckia, soybeans, and Maryland Mammoth tobacco. The effects of other environmental treatments as well as temperature indicate that the blossoming state results directly from the nature of the internal condition of the plant rather than from any specific external treatment.

Morning-glory plants with branches in different photoperiod environments blossomed throughout instead of giving a local response as is the case with some plants such as poinsettia, cosmos, and Maryland Mammoth tobacco.

Root development was not well related to the photoperiod treatment but rather to the foliage character of the top.

Orange Flare cosmos which blossoms in the long days of summer appears to be an example of an adaptation to temperature rather than to photoperiod.

If, as appears from the responses of several varieties of plants, adaptation is a genetic character, it deserves greater consideration than is now given it in plant-breeding work.

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STUDIES ON THE PHYSIOLOGY AND INHERITANCE OF FEATHERING IN THE GROWING CHICK[1]

By M. H. RADI, *graduate student*, and D. C. WARREN, *poultry geneticist, Kansas Agricultural Experiment Station*[2]

SCOPE OF THE PROBLEM

The term "feathering" as used in this paper refers to the extent to which the bird is clothed with feathers at the time it attains the broiler stage. In a study of this type, a wide range of factors is involved since feathering is a process of growth and development subject to the effects of numerous physiological and inherent agents. A consideration of the physiological nature of feathering requires investigation of the dietary, hormonal, and environmental factors.

From the marketing standpoint, good feathering is indispensable, if a profit is to be realized, for poorly-feathered broilers are subject to severe market discrimination.

STOCK USED

The birds used in all the studies which are to be discussed were a part of the flock carried at the Kansas Agricultural Experiment Station. Approximately 3,100 birds were utilized in a period of 4 years. Three strains of the Rhode Island Red breed referred to as the early-feathering, the well-feathering, and the poor-feathering were utilized.

The early-feathering strain is characterized by early-developing and rapid-growing feathers. The rate of feathering in these birds is similar to that commonly found in the birds of the Mediterranean breeds. This type of early feathering is a heritable, sex-linked, recessive characteristic. The strain was established by selection of early-feathering chicks in a strain known not to be homozygous for the sex-linked rate-of-feathering gene.

Both the well- and the poor-feathering stocks are known to be homozygous for the sex-linked dominant allel of early feathering. These two strains were the result of plus and minus selection for degree of broiler feathering in the usual late-feathering stock of the Rhode Island Red breed. An unselected strain of Single-Comb Rhode Island Red was used for most of the physiological studies.

FEATHER TRACTS

For the purpose of this study, 10 feather tracts are recognized. The name given to the tract is indicative of its location, which is shown in figure 1.

The differentiation among the birds used in this study was based on the condition of feather development at the end of the seventh or eighth week. An arbitrary scoring system was devised, and in esti-

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mating the feathering of a bird by this system each of the 10 feather tracts was individually graded. A maximum grade of three points was assigned to each of these tracts. In order that a tract might receive the perfect score of three points, the feathers growing thereon must have been sufficiently long and abundant to cover completely the whole area of the tract. A tract with feathers abundant but not quite long enough to exhibit a uniform surface was cut one point. A tract was cut two points if the feathers were sparsely scattered over the tract, many in the pin stage, and areas free from feathers were showing. If no feathers had appeared, and the down still persisted, a tract was given a score of zero. Thus a bird with well-developed feathers on all of its 10 tracts might make a maximum grade of 30

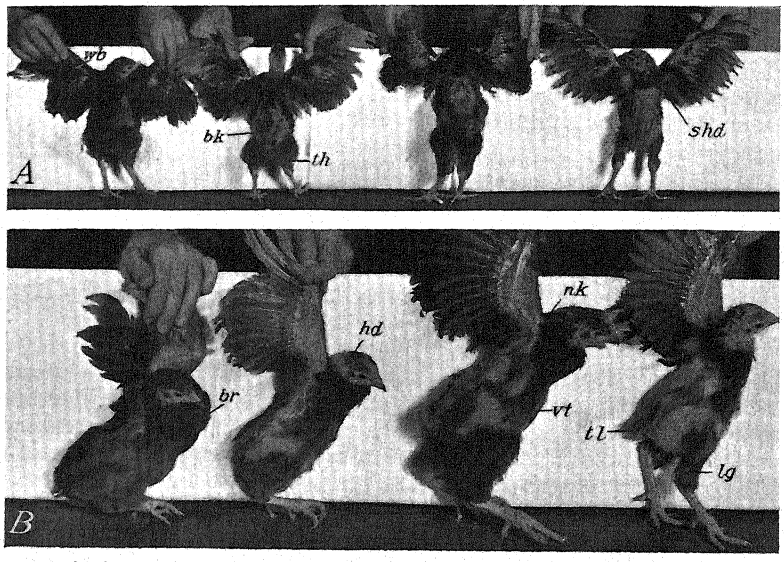


FIGURE 1.—Variations in degree of feathering and location of feather tracts: *A*, Dorsal view: *wb*, Web; *bk*, back; *th*, thigh; *shd*, shoulder. *B*, Lateral view: *br*, Breast; *hd*, head; *nk*, neck; *vt*, ventral; *tl*, tail; *lg*, leg.

points. Figure 2 shows representative skin samples from the back region of 7-week-old birds with scores of 0, 1, 2, and 3.

In the early work, the evaluation of feathering was made at 8 weeks of age. In the later investigations, however, it was made at 7 weeks. This change was deemed advisable, because it was found that the difference in the degree of feather growth among birds was obscured with advancing age. Thus, the variability among such birds was more strikingly evident at about the seventh week of age. Since considerable sexual dimorphism exists in relation to rate of feathering, it was impossible to select an age which would be optimum for both sexes. The age of 7 weeks was perhaps a little late for females, but a younger age would have been too early for the most satisfactory classification of males.

PHYSIOLOGICAL STUDIES

A number of different studies have been made during the course of this investigation with a view to ascertaining the physiological nature

of feathering. Dietary, hormonal, as well as environmental, agents have been studied in their relation to feathering. In the discussions which follow, the question of interrelationship among the various agents studied will be pointed out in the light of the results secured.

FEATHER GROWTH

The process of feather development has been studied by a number of workers; therefore, it is not necessary to go into the details at this time. However, a brief description of the more important steps leading to the formation of the feathers seems pertinent. Feathers first appear as small papillae on the skin of the embryo about the sixth day of incubation. These papillae, which arise from the epidermal layer of the skin, elongate and at the same time tend to sink

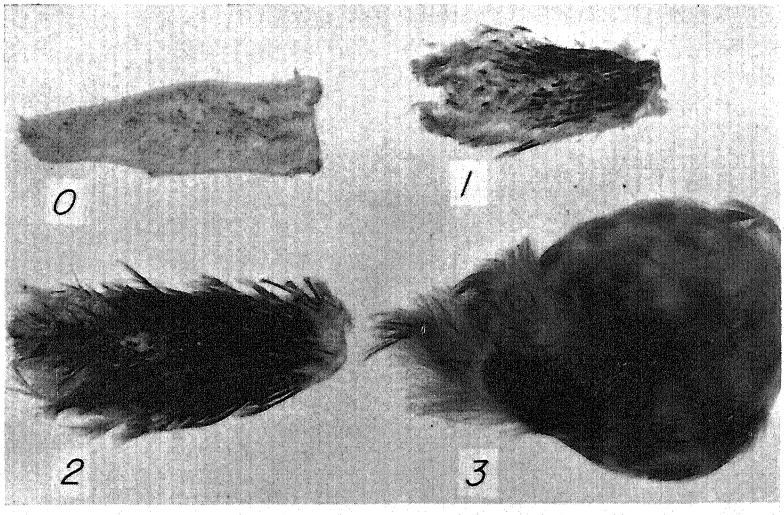


FIGURE 2.—Representative skin samples from the back region of 7-week-old birds with scores of 0, 1, 2, and 3.

below the surface into the dermis to form the feather follicles. According to Hosker (*8*),[3] the down and the definitive feathers arise in a similar manner from the stratum Malpighii of the skin and the papilla, respectively.

The definitive feather develops from the same papilla from which its downy predecessor had arisen, and at the time the definitive feather appears externally the interior end of the down is often attached to the tip of the newly formed feather.

A wide range of variation exists as to the time of appearance of feathers. Some chicks are practically fully clothed with feathers at 4 weeks of age, while others carry the juvenile down for as much as 12 weeks. The question arises as to what is the status of the feather in the birds in which this long-delayed emergence occurs.

Histological studies were made on skin from the backs of 7-week-old birds which were entirely devoid of feathers and comparison was made of skin from the back region of day-old chicks. The only difference observed was that the feather follicles in the 7-week-old bird appeared

[3] Reference is made by number (italic) to Literature Cited, p. 704.

deeper and larger than those in the day-old chick. This condition was evidently due to the relative thickness of the skin in the two birds rather than to further differentiation having occurred in the older bird.

The stage of development at which delay in feather development occurs seems to be the very initial stage in formation marked by the proliferation of the intermediate cells. No indication of this cellular proliferation was noted in the transverse skin sections of the 7-week-old birds which were studied. The histological picture of the inactivated feather germ to which the down is still attached, as found in a 7-week-old Rhode Island Red bird, is shown in figure 3. This figure

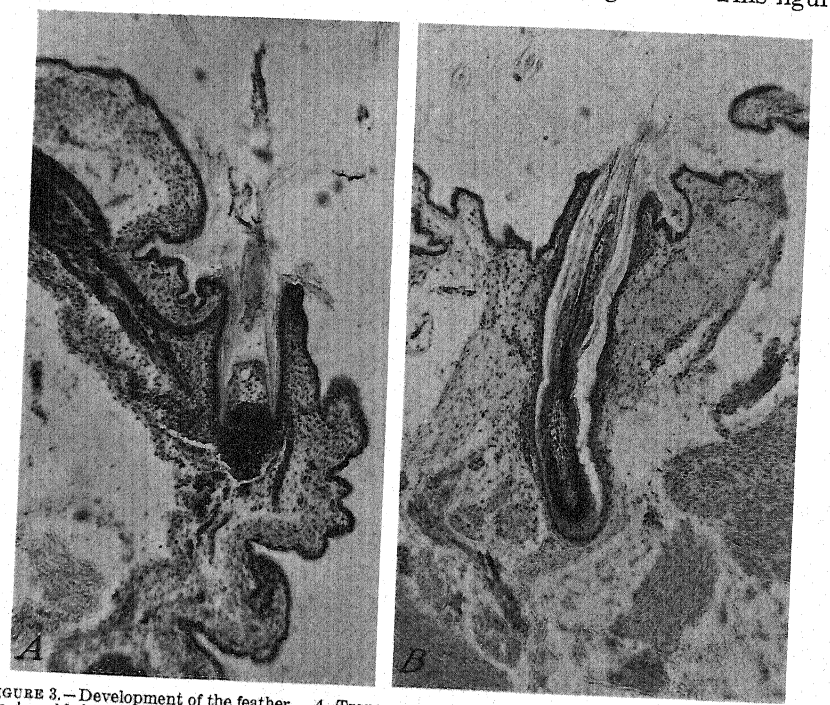


FIGURE 3.—Development of the feather. *A*, Transverse section of a feather follicle from the back region of a day-old chick. × 200. Implantation of the down in the follicle is evident. *B*, Transverse section of a feather follicle from the featherless back of a 7-week old chick. × 200. Note the persistence of the juvenile down and the similarity to the day-old chick follicle with respect to lack of cellular differentiation within the follicle. The feather follicles of this bird are deeper, because of its thickened skin.

also shows the details of the feather germ as it exists in a day-old chick of the same breed.

ORDER OF APPEARANCE OF FEATHERS IN THE TRACTS

The age at which the feathers begin to appear on the various tracts of a single bird differs as much as 8 weeks. The feathers located at the caudal end of a tract usually appear first and attain the greatest length.

A systematic study was made of the order of appearance of feather tracts. The chicks were examined at weekly intervals. The data obtained on the three strains of Rhode Island Reds used in this study are given in table 1. The data show the age at initial appearance of feathers in a tract and also variability in time of appearance. Similar data on the early- and late-feathered strains of White Leghorns are also shown in table 1.

The number of chicks in each sex in the various strains shown in table 1 ranged from 24 to 46. It should be kept in mind that the early-feathered Leghorns and the well- and poor-feathered Rhode Island Reds constitute the normal condition for these two breeds. The sex-linked early-feathered strains in the two breeds show an earlier development of feathers in most tracts than do the other strains. This is most strikingly exhibited in the tail tract.

TABLE 1.—*Time and order of appearance of feathers in the different tracts in early-, well-, and poor-feathered Rhode Island Reds and early- and late-feathered White Leghorns*

RHODE ISLAND REDS

Tract	Strain	Percentage distribution of birds on basis of age at time of appearance of tract															
		Males								Females							
		1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
Tail	Early	100								100							
	Well				13	61	18	4	4			8	63	29			
	Poor				13	33	7	20	27				20	20	27	27	6
Shoulder	Early	100								100							
	Well	12	36	36	12	4				17	79	4					
	Poor	6	27	7	27	13	13		7	33	20	13	27	7			
Thigh	Early	61	39							58	42						
	Well			60	32	8					13	83	4				
	Poor			20	47	20	7		6		7	40	20	27	6		
Breast	Early	13	87							21	79						
	Well			68	20	12					25	69	6				
	Poor			60	33	7					27	67	6				
Neck	Early		96	4							96	4					
	Well			4	40	36	16		4			13	70	16			
	Poor				13	33	7	27	20				20	40	40		
Back	Early		74	22	4						79	21					
	Well				8	46	29	13	4			8	46	42	4		
	Poor					7	13	40	40				13	27	33	20	7
Web	Early		92	4	4						92	8					
	Well				4	29	46	13	8				25	67	8		
	Poor						33	33	34				7	13	67	13	
Leg	Early		22	74	4						29	67	4				
	Well				71	17	12					17	75	8			
	Poor			7	73	20						33	60	7			
Ventral	Early			26	52	22						29	63	8			
	Well					41	41	14	4			4	21	63	12		
	Poor					7		53	40				6	27	40	20	7
Head	Early			9	83	8						29	63	8			
	Well				16	44	28	12				4	42	38	16		
	Poor				13	60	13	7	7				13	80		7	

WHITE LEGHORNS

Tail	Early	100								100							
	Late		12	6	70	12				7	33	33	27				
Shoulder	Early	100								91	9						
	Late		29	29	24	18				13	53	27	7				
Thigh	Early		89	11						9	82	9					
	Late		6	71	23						13	74	13				
Breast	Early		89	11						9	82	9					
	Late		6	71	23						20	73	7				
Neck	Early		11	83	6							100					
	Late			6	94							14	86				
Back	Early		11	72	17							82	18				
	Late			12	35	35	18					27	67	6			
Web	Early		6	72	22							82	18				
	Late				24	53	23					7	67	26			
Leg	Early			17	78	5						9	91				
	Late				88	12						13	80	7			
Ventral	Early			6	83	11							91	9			
	Late				41	53	6						93	7			
Head	Early			6	50	44							73	27			
	Late				24	76							60	40			

The order of appearance of the tracts varied somewhat in the different strains but usually the shoulder, thigh, and breast were the first regions to show feather development.

The various strains differ consistently enough to indicate that in addition to the well-known sex-linked pair, other genetic factors control the time of appearance of the adult type of plumage. The later classification as to feathering at 7 and 8 weeks is probably a measure of the same genetic differences noted here.

EFFECT OF BODY GROWTH ON RATE OF FEATHERING

The influence of body weight upon the rate of feathering at 8 weeks has been studied. It appears from the results of this study, which was carried out on unselected Rhode Island Red birds, that the influence is not great. The coefficient of correlation between body weight and grade of feathering was found to be 0.194 ± 0.013 and 0.055 ± 0.016 in 305 males and 297 females, respectively. These values are too low to be of any practical importance.

The analysis of data obtained from a similar study in 1933 gave somewhat higher correlation coefficients, these being 0.2398 ± 0.0359 in 312 males and 0.3833 ± 0.0336 in 292 females. Gericke and Platt (*6*) in work with Barred Plymouth Rocks secured a coefficient of correlation of 0.8120 ± 0.0109 between body weight and feather development when the chicks were 8 weeks old. It should be noted, however, that these investigators raised their birds on rations of different protein levels, which might have been a factor in securing such a high correlation value. In the present studies all the birds were given a normal ration and management. Jaap and Morris (*9*), working with a number of different breeds of fowl, reported a correlation coefficient of 0.23 between body weight and feathering at 8 weeks.

It thus appears from these studies that body weight and feathering as determined at the age of 8 weeks bear no close relationship.

SEX IN RELATION TO FEATHERING

Sexual dimorphism in rate of feathering was noted throughout this investigation. Table 1 gives a comparison of time of appearance of feathers in the various tracts of birds of the different strains studied. In this table is given the percentage of chicks showing each feather tract at ages ranging from 1 to 8 weeks. The data clearly demonstrate sexual dimorphism in the rate of feathering. A comparison of the males and females of any one strain shows an earlier appearance of the tract in the case of the females, or a higher percentage of the females showing feathering in a given tract at any period.

Studies made on the development of the embryonic feather papillae in connection with this investigation revealed that in the female chick embryo the feather papillae appear somewhat earlier than in the male embryo, the difference in time of appearance between the two sexes being only a matter of a few hours. On the basis of this fact, it was possible to distinguish the sex of the embryo by the relative development attained in its feather germs. The results of this phase of work are not yet published.

From the foregoing discussion, it is evident that feathering is greatly affected by sex, since the time of appearance of feathers and their rate of growth seem to be closely associated with sexual phe-

nomena. As a rule, the feathers of the female appear earlier and grow at a higher rate than those of the male.

MOLT IN RELATION TO BROILER FEATHERING

Molt is known to take place in the growing chick as well as in the adult bird. Marble (*14*) observed that at 6 weeks of age the White Leghorn female chicks showed considerable molt in neck, back, pelvic wing (thigh), and pectoral (breast) regions, while no molt was evident in the males of the same age. During the seventh and eighth weeks, he observed that this molt continued to show more distinctly, in the female alone. Dunn and Landauer (*5*), working on the Silver-Spangled Hamburg, reported that the process of molt occurs in a definite rhythm in a given feather tract and is almost continuous during growth. Warren and Gordon (*22*) noted that in the Rhode Island Red and Leghorn breeds, as well as in other domestic fowls, the juvenile remiges undergo at least one complete molt during growth.

The effect of molt in the growing chick on the feathering score as determined in this study was of interest. Eight Rhode Island Red and eight White Wyandotte chicks were dipped in a coloring solution of crystal violet when 3 weeks of age. The White Wyandottes were used primarily because of the advantages of dyeing a white plumage.

Weekly observations were made on all the feather tracts of these chicks to ascertain whether molt had occurred. The fact that in the majority of individual tracts the feathers make their appearance in a definite succession, namely, the postero-anterior direction, was of some aid in the search for the molted feathers. The presence of an undyed feather among the feathers of dyed tips was indicative of molt.

At the age of 6 weeks, by which time practically all the feather tracts had made their appearance, the chicks were dipped in a green dye. The green color, however, was not sufficiently dense to mask the purple used in the previous dyeing.

The results of this study indicate that molt previous to 9 weeks of age is not a factor of importance in broiler feathering. No indication of molt was observed during the first 7 weeks of this experiment, and only slight evidence of molt was noted at 9 weeks. It is clear that the first molt in Rhode Island Reds and Wyandottes takes place later than in the Leghorns in which Marble (*14*) found molt occurring at 6 weeks of age. The findings also show that molt does not materially affect the results of this study.

RELATION OF DEGREE OF FEATHERING AT EARLIER AGES TO THE FEATHERING SCORE

Length of Wing Feathers of Day-Old Chicks and the Feathering Score

One of the problems considered during the course of this investigation was to determine the relationship between the growth rate of the primary wing feathers and the feathering condition of the bird at 8 weeks. The primary wing feathers are the first definitive feathers to appear on the chick of the domestic fowl. As a rule, these feathers are present when the chick hatches.

All the Rhode Island Red chicks that were hatched during the season of 1934 were examined on the day of hatching to determine the relative length of the wing primaries. This stock did not carry the sex-linked early-feathering factor. These chicks were classified

into two arbitrary groups according to the length of their primary feathers. Those which had long primaries (approximately one-half inch) comprised one group and those which had short primaries (approximately one-third inch) comprised the other. At 8 weeks of age, the degree of feather growth was determined according to the scoring scale previously described.

From the results secured, the length of primary wing feathers at hatching does not seem to bear any relation to the 8-week feathering condition. The 8-week mean feathering scores of 210 males and 220 females which had, as baby chicks, long primaries, were 23.8 and 27.6 points, respectively. On the other hand, the mean scores of 95 males and 77 females designated as having short primaries when they were 1-day old were 22.2 and 27.0 points, respectively. The lack of difference between these two groups of birds may be ascribed to the influence of the time elapsing since hatching. The chicks that emerged from the shell first are most likely the ones which were classed as having longer primaries, while those that hatched late are probably the ones which had short primaries and, therefore, were designated as such. At any rate, the results indicate that this measure of variability is not to be relied upon for the purpose of prediction.

Number of Primary Wing Feathers in Day-Old Chicks and the Feathering Score

The number of primary wing feathers present in day-old chicks was studied in relation to 7-week feathering score. As previously stated, the wing primaries are the first definite feathers to appear on the chick. The number of these feathers present at the time of hatching is subject to considerable variation, even among birds of the same strain.

The observations upon which this study was based were made on a group of Rhode Island Red chicks hatched from an unselected population known to carry the sex-linked late-feathering factor. At the time of hatching, the chicks were examined and the number of primary wing feathers present was recorded. These chicks were reexamined at the age of 7 weeks to determine the degree of development attained by their feathers. The data secured from observations made on 731 birds, including 271 males and 460 females, were tested for the degree of association between the number of primary wing feathers present at the time of hatching and the 7-week feathering score. The coefficient of correlation secured was 0.2038 ± 0.039 in the males, and 0.2774 ± 0.029 in the females. These correlation values indicate that the two variables considered are significantly associated with each other. As a practical basis of prediction, however, the value of correlation is low.

Length of Wing Feathers of 13-Day-Old Chicks and the Feathering Score

Another point of interest is the relationship between the length of wing feathers at 13 days of age and the 8-week feathering score. In this experiment, the birds were examined on the thirteenth day of age and the length of the wing feathers was noted in comparison with the length of the body. The length of the wing feathers at this stage of development varied considerably. Some birds had wings the tip of which when folded reached approximately half the

length of their bodies, while in other birds these feathers were as long as seven-eights of the body.

The birds were classified into four groups, according to the relative length of their wings. These groups were designated as ½, ⅝, ¾, and ⅞, in comparison of the wing with body length. At the termination of the experimental period of 8 weeks, and after the birds had been graded according to the scoring scale previously mentioned, they were classified into these four different groups based on wing length and the mean 8-week feathering score of each of the four groups was determined. The results of the classification of 305 males and 297 females are summarized in table 2.

TABLE 2.—*Relation of length of primaries in 13-day-old chicks to the degree of feathering at 8 weeks of age*

Sex	Mean 8-week feathering score for birds having indicated length of wing at 13 days			
	½ body length	⅝ body length	¾ body length	⅞ body length
Females	26.38	27.66	28.27	29.00
Males	22.45	23.93	26.85	

The data in this table show that the longer the wing in relation to length of body at 13 days of age, the better the feathering at 8 weeks, and vice versa. This fact might be utilized as an index for evaluating the future feathering of the bird.

In connection with this study, the coefficient of correlation between 3-week and 8-week feathering was also determined on 254 males and 272 females. The correlation values were 0.5896 ± 0.0276 and 0.5840 ± 0.0269 in the males and females, respectively. These values indicate a rather high degree of association between the feathering condition of the two ages considered.

NUTRITIONAL STUDIES

No exhaustive nutritional studies were planned; only those dietary factors were investigated that seemed to bear directly on the problem. The nutritional tests were made with Rhode Island Reds, previously unselected for feathering condition. The type of feathering found in these birds is very similar to that commonly present in most of the larger breeds of fowl. This stock was known to be homozygous for the sex-linked late-feathering factor. Numerous studies (*15*, *17*, *19*, *24*) have been made of the vitamin A requirements of the chick, but little attention has been given to the influence of the level of this vitamin on chick feathering. Three lots of birds, each consisting of 49 Rhode Island Red chicks, were used in this experiment. The chicks in this and other nutrition experiments were kept in battery brooders. To each of the three lots the following basal ration was fed: 37 pounds ground white corn, 16 pounds wheat shorts, 16 pounds wheat bran, 16 pounds ground oats, 15 pounds meat meal, and 1 pound salt.

In addition to this ration, vitamin A was supplied in the form of a commercial concentrate which, according to the manufacturer, con-

tained 1½ million International units of vitamin A per pound. The number of units of vitamin A fed to each of the three lots was 1,160, 3,311, and 5,291 per pound of feed. To avoid the loss of vitamin A potency only enough mash was mixed at a time to carry a lot of chicks for a week. It may be noted that the use of subminimal quantities of vitamin A would have been inadvisable in an experiment of this nature, since the retardation of body growth might affect the growth of feathers.

Vitamin D was also supplied the chicks in the form of concentrate.

The results of this experiment (table 3) seem to indicate that vitamin A in the quantities here used is not an important factor in feather growth. The analysis of variance (*18*) showed that variations in feathering due to the different levels of vitamin A used were too low in the female to be of significance. In the males, however, the variations closely approached the point of significance. The ratio of the variance between the means of the three vitamin levels to that of the experimental error, commonly designated as F, is 2:10, while the expectation of random sampling on the basis of 5 times in 100 trials is 2:14. Therefore, vitamin A in the quantities fed in this study was of questionable value in promoting feather growth.

TABLE 3.—*The average 7-week feathering grade and body weight of 3 lots of chicks fed different quantities of vitamin A*

Lot No.	Vitamin A per pound of feed	Males			Females		
		Chicks	Mean feathering score	Mean body weight	Chicks	Mean feathering score	Mean body weight
	International units	*Number*		*Grams*	*Number*		*Grams*
1	1,160	22	6.4	332.7	18	9.0	323.8
2	3,311	26	7.1	352.7	19	9.9	323.7
3	5,291	20	8.0	343.5	20	8.7	295.0

Grains and their byproducts make up the major part of the poultry ration. It is therefore desirable to know the extent to which the various grains affect the growth of feathers at the broiler stage of development.

Branion (*1*) investigated the role of corn, wheat, oat groats, and barley in poultry nutrition and found that chicks raised on a high intake of corn did not grow feathers over the back and their feather development was generally retarded. In a study to determine the comparative value of ground oats and wheat products in chick feeding, Wilcke (*23*) reported that the chicks raised on a ration consisting of 72 percent of ground oats had normal growth and excellent feathering. He also reported that the chicks raised on a ration consisting of 72 percent of ground yellow corn made poor growth and their feathering was of poor quality.

Three of the most widely used grains in poultry feeding, namely, corn, wheat, and oats, were chosen for this test. Four groups of birds, each consisting of 56 Rhode Island Red chicks, were used. One group each was fed exclusively on either corn, wheat, or oats. The fourth group was given a combination of the three grains. A basal mixture consisting mainly of concentrates was fed to all chicks in

addition to these grains. This mixture consisted of 7 parts meat scrap (high grade), 8 parts dried buttermilk, 5 parts alfalfa leaf meal, 1 part salt, and 1 part cod-liver oil.

Equal quantities of the basal mixture were used in making up the mash fed to the four lots of chicks. Likewise, the total crude protein in each of the four rations was kept equal and uniform throughout the test. The composition of the four rations used in this study is given in table 4 and a summary of the results secured is given in table 5.

TABLE 4.—*Composition and the total crude-protein content of rations used for comparison of influence of various cereal grains on feathering*

Ingredient	Rations used for—							
	Lot No. 1		Lot No. 2		Lot No. 3		Lot No. 4	
	Constituents	Crude-protein content	Constituents	Crude-protein content	Constituents	Crude-protein content	Constituents	Crude-protein content
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Basal mixture	22.0	8.34	22.0	8.34	22.0	8.34	22.0	8.34
Ground yellow corn	70.5	6.55					23.5	2.34
Ground whole wheat			78.0	9.59			26.0	3.20
Ground oats					78.0	9.75	26.0	3.25
Corn gluten meal	7.5	2.86					2.5	1.02
Total	100.0	17.75	100.0	17.93	100.0	18.09	100.0	18.15

TABLE 5.—*Effects of various grain rations shown in table 4 on feathering and growth*

Lot No.	Grain	Birds	Sex	Mean feathering score	Mean body weight
		Number			*Grams*
1	Corn	34	Male	7.0	388.2
		21	Female	10.3	359.5
2	Wheat	26	Male	7.9	380.3
		21	Female	10.7	346.2
3	Oats	20	Male	8.8	447.5
		24	Female	10.0	375.8
4	Mixture of corn, wheat, and oats.	26	Male	7.5	436.5
		20	Female	10.6	407.5

Table 5 shows that the males of lot 3, raised on the oat ration, made the highest feathering score and gained the most in body weight. The females of the same lot did not show any improvement in their feathering over those of the other lots, but they showed a better gain in body weight than was made by lots 1 and 2. Lot 4, for which a mixture of the three grains was used, made the most rapid gain in body weight.

Lot 3 had the highest mortality, 23 percent at 7 weeks of age. Mortality in lots 1, 2, and 4, was 1.8, 16.0, and 17.8 percent, respectively. Some perosis occurred among the chicks which were on a corn ration.

The data were subjected to analysis of variance, and the variations in feathering due to differences in the composition of ration were not large enough to have statistical significance. The ratio of the variance between the means of the 4 rations to that of the experimental

error is 1.84, while the expectation of random sampling on the basis of five times in 100 trials is 2.7.

In the nutritional studies, the effects of vitamin G (B_2) and protein level of the ration on the growth and development of feathers were also tested. In this test, four rations in which the percentage of protein varied from 11 to 25 were used. Widely different ranges of vitamin G were maintained in these rations. Although as a result of disease, the number of birds utilized in this test was not sufficiently large to permit any definite statement regarding the role of either protein or vitamin G in the growth of feathers, the results seemed to indicate that both of these nutrients are of some importance in this respect. The variations in feathering due to feeding different levels of protein were more significant than those ascribed to the vitamin G content of the ration.

Two other studies of nutritional interest were also made for the purpose of testing the effects of magnesium carbonate and cystine on the growth of feathers. In neither of the two tests was the number of birds large enough to warrant a definite conclusion. However, the results seemed to indicate that feeding a standard poultry ration supplemented with 3 percent of magnesium carbonate or 0.5 and 1 percent of cystine in no way influenced feather growth.

ENDROCRINOLOGICAL STUDIES

Activity of the Thyroid Gland in Relation to Feathering

Various studies (*4*, *12*) have been attempted to discover the role of the thyroid of the fowl in relation to body size, egg production, sexual activity, plumage structure, and pigmentation of feathers, as well as to many other physiological phenomena. Schwarz (*16*) observed that in thyroidectomized birds, feather growth was inhibited. He concluded that the increase in thyroid activity accelerates feathering. Hardesty (*7*), who studied the effect of thyroxine injections upon the feathers of guinea fowl, has stated that thyroxine, even in small doses, abruptly stimulates the germ lying quiescent in the follicle. She also noted that without exception feathers treated with thyroxine reach maturity at an earlier age than do controls from the same follicles. Chaudhuri (*2*) found that the iodine content of the thyroid of sexually immature males is lower than that of the thyroid of mature males. He, however, found no indication of sexual dimorphism with respect to the iodine content of this gland. Cruickshank (*3*) corroborated the findings of Chaudhuri regarding the increase in iodine content in the thyroid of the male with the advance in age. In his biochemical studies on the epidermal structures of mammals and birds, Kosjakoff (*11*) reported that generally more iodine is found in the feathers and hairs of the female than in those of the male.

Two groups, an experimental and a control, each consisting of 20 late-feathering Rhode Island Red chicks were used in this investigation. Since it was desirable to have the two groups of chicks as nearly identical as possible, they were selected as pairs, each pair chosen to match as perfectly as possible in body size and degree of feather growth on the various tracts. At the age of 3 weeks a condition of hyperthyroidism was artifically induced in the chicks of the experimental group by injecting into the pectoral muscles of each chick 0.5 mg of crystalline thyroxine dissolved in 0.5 cc of distilled water. The

experimental and control chicks were kept together in an electric battery brooder, insuring similar environmental conditions.

Preliminary results showed that there was no difference with respect to feather growth among the birds repeatedly injected with thyroxine and those that received a single injection.

The results of this study are summarized in table 6. The experimental group made a higher mean feathering score than the controls. In only one case, male No. 2806, did the administration of thyroxine fail to induce improvement in the feathering over that found in the other member of the pair. The mean feathering score of the birds injected with thyroxine was 14.8 points for the males and 25.2 points for the females. On the other hand, the birds which received no thyroxine scored 10.0 and 20.0 points for the males and the females, respectively. With respect to body weight birds of the same sex in the experimental and control groups were very similar. It thus appears that the increased thyroid activity of the bird leads to a stimulation in the rate of its feather growth.

It is to be concluded from this study that the administration of thyroxine into Rhode Island Red chicks at the age of 3 weeks caused the feathering of both male and female chicks to be accelerated.

TABLE 6.—*Influence of thyroxine injection on feather growth*

Experimental group				Control group			
Sex and chick No.	3-week weight	7-week weight	Feathering score	Sex and chick No.	3-week weight	7-week weight	Feathering score
Male:	*Grams*	*Grams*		Male:	*Grams*	*Grams*	
2797	160	410	14.5	2868	160	560	11.0
2804	170	540	12.5	2786	180	500	12.0
2806	100	340	3.0	2833	100	350	9.0
2811	190	580	24.5	2852	190	520	12.0
2820	170	470	19.0	2860	170	540	11.5
2838	190	550	17.5	2874	190	620	17.0
2844	185	480	17.0	2828	190	600	9.0
2845	150	440	10.0	2863	160	560	8.5
2851	180	600	16.5	2861	180	450	13.0
2866	100	450	14.0	2876	90	340	6.5
2867	130	420	17.0	2826	130	440	7.5
2871	150	550	13.0	2878	150	300	3.0
Mean		485.5	14.8	Mean		481.6	10.0
Female:				Female:			
2788	180	550	24.5	2791	180	530	18.5
2793	180	420	20.0	2792	190	580	18.0
2799	170	370	28.0	2882	160	520	23.5
2810	180	600	25.5	2884	190	460	21.0
2812	160	440	23.0	2842	170	340	15.0
2832	220	560	27.0	2897	210	640	24.5
2856	200	540	27.0	2886	190	520	17.5
2996	210	520	26.5	2873	210	560	22.5
Mean		500.0	25.2	Mean		518.8	20.0

EFFECT OF IODINE ON GROWTH OF FEATHERS

The effect of iodine on the growth of feathers was tested by feeding 21 birds, of which 14 were males and 7 were females, a standard ration to which potassium iodide was added at the rate of 1.5 g per 100 pounds of feed. The mean feathering score of the males at the age of 7 weeks was 13.5, while that of the females was 21.6. In another group of birds which were kept as controls 9 males had a mean feathering

the number of birds employed was not large enough to warrant definite conclusions with respect to the role of iodine in the growth of feathers, the results indicated that iodine has some stimulating effect on feather growth. However, this effect was more markedly expressed in the male than in the female.

EFFECT OF ENVIRONMENT ON GROWTH AND DEVELOPMENT OF FEATHERS

HUMIDITY

It is a common belief among poultry producers that high humidity in the brooding room is conducive to better feathering. Although high humidity is recommended as one of the requirements for successful brooding, no experimental evidence in support of this view has been found. An experiment was accordingly undertaken to ascertain the extent to which humidity is a factor in broiler feathering.

One lot of 60 Rhode Island Red chicks was raised in a relatively dry basement room while another comparable group was housed in a humid one. All chicks were kept in a frame structure made of wire and elevated about 3 feet from the floor to permit free circulation of air. In the dry room, no liquid material other than water was present and droppings were removed daily to restrict evaporation. The floor and walls of the humid room were thoroughly moistened several times during the day and evening. A fine mist spray operating continuously in the room saturated with water a large piece of burlap hanging from the ceiling. Temperature and humidity in both rooms and outdoors were recorded each morning and afternoon. A sling psychrometer was used for taking humidity readings. Efforts were made to maintain the same temperature in the two rooms. The mean morning and afternoon temperatures of the humid room for the duration of the experiment were 79.4°±0.56° and 83.3°±0.40° F., while those of the dry room were 81.5°±0.49° and 84.4°±0.39°.

The mean relative humidity in the humid room for the duration of the experiment was found to be 72.2 percent in the morning and 69 in the afternoon, as compared with 51.8 and 50.7 in the dry room. The outside atmosphere was more humid than that of the dry room, but considerably less so than that of the wet room.

All chicks were fed a growing ration containing about 18 percent of protein, and except for humidity were treated alike. At the close of the seventh week the birds were weighed and graded for feathering.

The results of this experiment (table 7) indicate that higher humidity favors feather growth but that it has no effect on body weight.

TABLE 7.—*Influence of humidity on feathering*

Humidity	Mean relative humidity		Mean temperature		Birds	Sex	Mean feathering score	Mean body weight
	A. M.	P. M.	A. M.	P. M.				
	Percent	*Percent*	*° F.*	*° F.*	*No.*			*Grams*
High	72.2	69.1	79.4	83.3	31	Male	12.3	490.0
					22	Female	18.2	453.1
Low	51.8	50.7	81.5	84.4	32	Male	11.1	464.6
					20	Female	15.8	459.5

The analysis of variance shows that the ratio of the variance between the means of the two groups of birds to that of the experimental error, commonly referred to as F, is 6.02, while the expectation of random

sampling from a homogenous population is only 3.94, thus indicating that the difference in feathering is statistically significant.

TEMPERATURE

Although no critical experiments on the subject could be found, one frequently finds the statement that too high brooding temperatures tend to retard feather growth. Kleiber and Dougherty (*10*) have conducted an extensive investigation on the effect of environmental temperature on the utilization of food energy in the White Leghorn chick. They reported that the daily rate of growth was increased when the environmental temperature was decreased. Likewise, the chicks raised at 21° C. were found to have a much higher basal metabolism than those raised at 40°. In another study made by Landauer (*13*) on the relation of thyroid gland activity to environmental temperature in the Frizzle fowl, it is reported that a change in the environmental temperature resulted in a corresponding change in the activity of the thyroid gland, which consequently led to similar changes in the metabolic rate of the bird.

Two groups of 90 Rhode Island Red chicks were kept in adjacent basement rooms of equal size. The chicks in the high-temperature room were supplied heat from an electric hover placed on the floor, and in addition the entire room was heated by a gas stove to a temperature averaging 85° F. throughout the period of the experiment. The chicks in the low-temperature room were provided an electric hover but no supplementary heat. Thus the room was kept fairly cool (68°) except under the hover, which remained just warm enough to supply the chicks with the necessary heat. In the low- temperature room the temperature under the hover was gradually reduced and during the last half of the experiment the current was shut off. In this room the mash hoppers and the water fountains provided were placed at a distance from the hover in order to induce the chicks to spend as much time as possible away from the heat of the hover. Temperatures were recorded twice daily 6 inches from the floor both under and outside the hovers.

The two lots were fed the regular Kansas State College growing mash and a record of the amount of feed consumed by each lot was kept. At the close of the seventh week, individual weights were taken and the degree of feathering was evaluated.

The results of this experiment (table 8) indicate that low temperature has a stimulating influence on feather growth. The influence of the environmental temperature was more evident in the females, the improvement in feathering of females being 28.5 percent and that of males 19 percent.

The analysis of variance shows that the variation in feathering due to differences in environmental temperature is highly significant. The ratio of the variance between the means of the two groups to that of the experimental error is 47.3, while the expectation of random sampling on the basis of once in 100 trials is only 6.81. It is to be noted that feathering here was much better in both groups than in other experiments. This might have been due to the fact that the birds used in this study were given access to the entire floor of the room, whereas in the other experiments the chicks were raised in battery brooders.

TABLE 8.—*Influence of brooding temperature on feather and body growth*

Temperature	Mean temperature		Birds	Sex	Mash consumed per chick	Mean feathering score	Mean body weight
	Hover	Room					
	°F.	°F.	*Number*		*Pounds*		*Grams*
High	88.6	85.0	44	Male	2.74	12.1	571.6
			42	Female		15.4	506.0
Low	81.9	68.3	36	Male	3.01	14.4	563.3
			41	Female		19.8	541.2

Mortality was somewhat higher among the chicks raised in the cold room than among those in the warm room, being 14 and 4 percent, respectively. The increased mortality among chicks raised in the cold room may have been due to the rather cool environment in which they were compelled to live. The chicks in the cold room consumed more feed than did those in the warm room. This observation confirms the findings of Kleiber and Dougherty (*10*). The chicks in the cold room were more active than those in the warm room. In a number of instances the birds in the cold room actively engaged in a search for food, while those in the warm room squatted lazily on the floor. Thus increased exercise may have been responsible for increased food consumption and a resulting acceleration of metabolism.

CONCLUSIONS

Vitamins A and G have been found to be of questionable value in promoting the growth of feathers. Oat feeding produced feathering of questionable superiority as compared to corn and wheat.

Low brooding temperature and high humidity were shown to be of importance in bringing about better feathering. Landauer (*13*) has found that a condition of hyperthyroidism prevails in chicks kept at a low temperature, and since an induced hyperthyroidism results in better feathering as found in the experiment on thyroxine, it appears that the improved feathering in the chicks raised at a low temperature bears a relation to the functioning of the thyroid gland in these birds. The feeding of iodine seemed to make some improvement in feathering.

There is evidence in several experiments that the males are more responsive than the females to changes in diet, when measured by feather growth. There is probably no sexual dimorphism as to responsiveness, but this apparent difference is due to the fact that at the age of examination the males were at a more critical stage. The females were probably a little beyond this stage and the results were less evident. Body molt was not a factor of importance in feathering as considered in these studies.

GENETIC STUDIES

The genetic phase of the work consisted of studies of the hereditary nature of feathering in its diverse types as these occur in the Rhode Island Red and White Leghorn breeds.

Since the hereditary behavior of early and late feathering, ordinarily present in the so-called light and heavy breeds of the domestic fowl, respectively, is well understood, particular attention was given throughout this work to the nature of variations found in a strain of the Rhode Island Red breed, known to be homozygous for the dominant sex-linked late-feathering character. An attempt also was made to establish by selection in opposite directions, extreme types of

feathering in the Rhode Island Red. Various crosses have been made between the different types of feathering found in the Rhode Island Red and White Leghorn to ascertain whether feathering in the two breeds has the same genetic basis.

BREEDING LINES

Three strains of the Rhode Island Red breed, namely, the early-feathering, the poor-feathering, and the well-feathering, and two strains of the White Leghorn breed, early- and late-feathering, were used in this work. The establishment of these strains was accomplished through breeding and selection over a period of years.

The White Leghorn fowl is ordinarily classed as an early-feathering bird, in which feather growth is known to be controlled by a recessive, sex-linked gene (*20*). The so-called late-feathering White Leghorns, carrying the dominant allel of early-feathering, do not commonly exist in this breed. This strain was developed at the Kansas station by first outcrossing to Barred Plymouth Rocks and then repeatedly backcrossing the resulting offspring to White Leghorns. By such a procedure it was possible to establish a strain breeding true for nearly all of the Leghorn characteristics but possessing the sex-linked late feathering of heavy breeds.

The Rhode Island Red breed is ordinarily homozygous for the sex-linked late-feathering. However, considerable variation occurs in the rate of feathering of Rhode Island Reds carrying the late-feathering factor, and these variations were the sources of material for selection in the well- and poor-feathered strains. There was considerable overlapping of extremes of these two strains, but as data presented later will indicate, significant differences were established by selection.

The so-called early-feathering Rhode Island Reds were of a strain homozygous for the sex-linked early-feathering gene. Such individuals occasionally are found in pure-breeding stock of Rhode Island Reds and the mating of such individuals established this strain. The early-feathering Rhode Island Reds were much like ordinary White Leghorns in the rate of feather growth. In contrast to most other Rhode Island Reds, the chicks of both sexes are practically fully covered with feathers at 4 weeks of age.

The well- and poor-feathered Rhode Island Reds and the late-feathered White Leghorns have the same genetic constitution in that each carries the sex-linked late-feathering gene. The early-feathered Rhode Island Reds carry the sex-linked recessive gene for early-feathering which is characteristic of ordinary White Leghorns. Any differences in feathering of these strains at the broiler stage gradually disappear and are entirely eliminated at sexual maturity.

INFLUENCE OF SELECTION IN WELL- AND POOR-FEATHERED STRAINS OF RHODE ISLAND REDS

During the course of this study, an attempt was made to produce extreme types of well- and poor-feathered Rhode Island Red birds. This was done by selecting as breeders the best-feathered birds in the well-feathered strain and the slowest feathering ones among the poor-feathered strain. The progeny test was emphasized in the selection work. Not only was the individual feathering graded, but the family averages were also given consideration in the choice of breeders in each generation. In order to maintain satisfactory viability in the

stock, some attention was given to the vigor of birds mated and in some instances sacrifices were made in feathering grade to have vigorous birds in the matings.

In 1933 and 1934, the data on feathering were secured when the birds were 8 weeks old. It was decided, however, that 7 weeks was a more critical age and the later data were based on records taken at that age. Therefore, a direct comparison between the results of selection obtained in 1933 and 1934 and those obtained in 1935 and 1936 is not possible.

The results obtained in 1933 showed that selection was of slight effect in bringing about a marked difference in the two strains. During this year four matings were made, two of which were well-feathered while the other two were poor-feathered. The mean feathering scores of the offspring secured from the well-feathered matings were 12.8 and 11.8 for the males and 20.5 and 17.4 for the females, as compared with 11.7 and 12.3 for the males and 17.4 and 16.6 for the females of the two poor-feathered matings. The slight difference between the mean scores of the two selected lines is probably due to the fact that selection in 1933 was accomplished by choosing from mass populations birds showing either extremely good or poor feathering. For the 3 years which followed, selection was carried out on the basis of both individual feathering score and progeny performance. The scores made in 1933 are not strictly comparable to those made in 1934, since the junior author did the scoring the first year. In the succeeding years all scoring was done by the senior author and every effort was made to keep the standards uniform.

A summary of the results obtained in 1934, 1935, and 1936 is presented in table 9. It will be noted that the score of feathering made at 8 weeks of age is considerably higher than that at 7 weeks of age. In 1934, when feathering was evaluated at the age of 8 weeks, 2 well-feathered matings produced 342 birds and 3 poor-feathered matings produced 259 birds which had considerably lower mean scores. Although these values cannot be compared with those of 1935 and 1936 because of the age factor previously mentioned, nevertheless selection during 1934 must have accomplished something in segregating lines differing in their genetic make-up. The difference in score of the two strains is statistically significant.

TABLE 9.—*Results of selection within the well- and poor-feathered strains of Rhode Island Reds for a period of 3 years*

Year	Males			Females		
	Birds	Mean feathering score	Mean weight	Birds	Mean feathering score	Mean weight
	WELL-FEATHERED STRAIN					
	Number		*Grams*	*Number*		*Grams*
1934 [1]	169	25.4±0.18	521.7± 5.98	173	28.9±0.10	475.6± 5.15
1935	77	9.2± .25	312.5±16.61	54	16.0± .44	306.3±20.67
1936	50	9.0± .31	337.0± 8.18	112	14.0± .24	325.2± 4.26
	POOR-FEATHERED STRAIN					
1934 [1]	135	20.6±0.27	509.7± 4.83	124	25.4±0.24	466.9± 4.96
1935	59	5.1± .18	330.0± 6.63	36	8.4± .40	316.9± 7.65
1936	58	4.4± .21	325.2± 8.01	75	6.8± .25	300.3± 5.84

[1] In 1934 scoring was done at 8-weeks of age while in later years these records were taken at 7 weeks.

The results for the year 1936 show that selection to obtain better feathering in the well-feathered strain did not effect any improvement over 1935 in either sex, as judged by the mean feathering scores of the years concerned. In 1935 and 1936, three well-feathered matings were made each year. The individual mean feathering scores of the three matings made in 1935 were 8.7, 9.0, and 9.5 points for the males and 23.4, 14.3, and 16.1 points for the females, respectively, while the means for the three 1936 matings of this strain were 9.1, 9.0, and 8.8 points for the males and 13.4, 14.5, and 14.2 points for the females, respectively.

With respect to the influence of selection in the poor-feathered strain, the results presented in table 9 show that the mean feathering scores of both sexes were lower in 1936 than they were in 1935, indicating some effects of selection in the last year's matings. The individual mean feathering scores for the three matings of 1935 were 4.8, 6.1, and 4.9 points for the males and 8.0, 11.2, and 7.6 points for the females, respectively, while those of the four matings made in 1936 were 4.8, 2.6, 3.6, and 6.5 points for the males and 5.4, 5.0, 7.6, and 9.4 points for the females, respectively. In each generation of selection the differences in feathering score of the well- and poor-feathered strains are very definite and statistically significant, indicating that selection had segregated two strains which differed genetically. Representative groups (1936 hatch) of the well-feathered and poor-feathered males at 7 weeks of age are shown in figure 4, *A* and *B*, respectively. Representative females from the well- and the poor-feathered strains at the same age are shown in figure 5, *A* and *B*.

Selection as practiced in these studies necessitated a considerable amount of inbreeding in each of the strains. Despite the fact that mating of closely related individuals was avoided, there appeared during the breeding season of 1935 definite signs of reduced viability among the progeny of the selected lines. There was no significant decline in size of the birds in the selection strains in 1936 over 1935 and it seems that inbreeding was not responsible for the lowered feathering score in the poor-feathered strain.

It is to be concluded that selection for a period of 4 years was effective in segregating strains which differed in their genetic constitution for rate of feathering and that most of the results were accomplished in the first 2 or 3 years of selection.

MATINGS OF THE WELL- AND POOR-FEATHERED STRAINS

F_1 Generation Results

During the 1935 breeding season, reciprocal crosses involving well- and poor-feathered Rhode Island Red strains were made. Each male used was mated to females of his own strain as well as to those from the other strain. This tended to eliminate effects of the individuality of the birds used as parents, making birds of the selection strains for 1935 half brothers and sisters to the hybrids.

A summary of the results obtained from reciprocal crosses between birds of the well- and poor-feathered strains, as well as the selection results for the same year, are given in table 10. In both sexes the mean feathering scores obtained from the reciprocal crosses were virtually



FIGURE 4.—Seven-week-old males from the fourth generation of selections of the well-feathered strain (*A*) and the poor-feathered strain (*B*).

identical, the two groups of hybrid males scoring 8.1 and 8.8 while the females scored 13.8 and 13.1. The distribution of male feathering

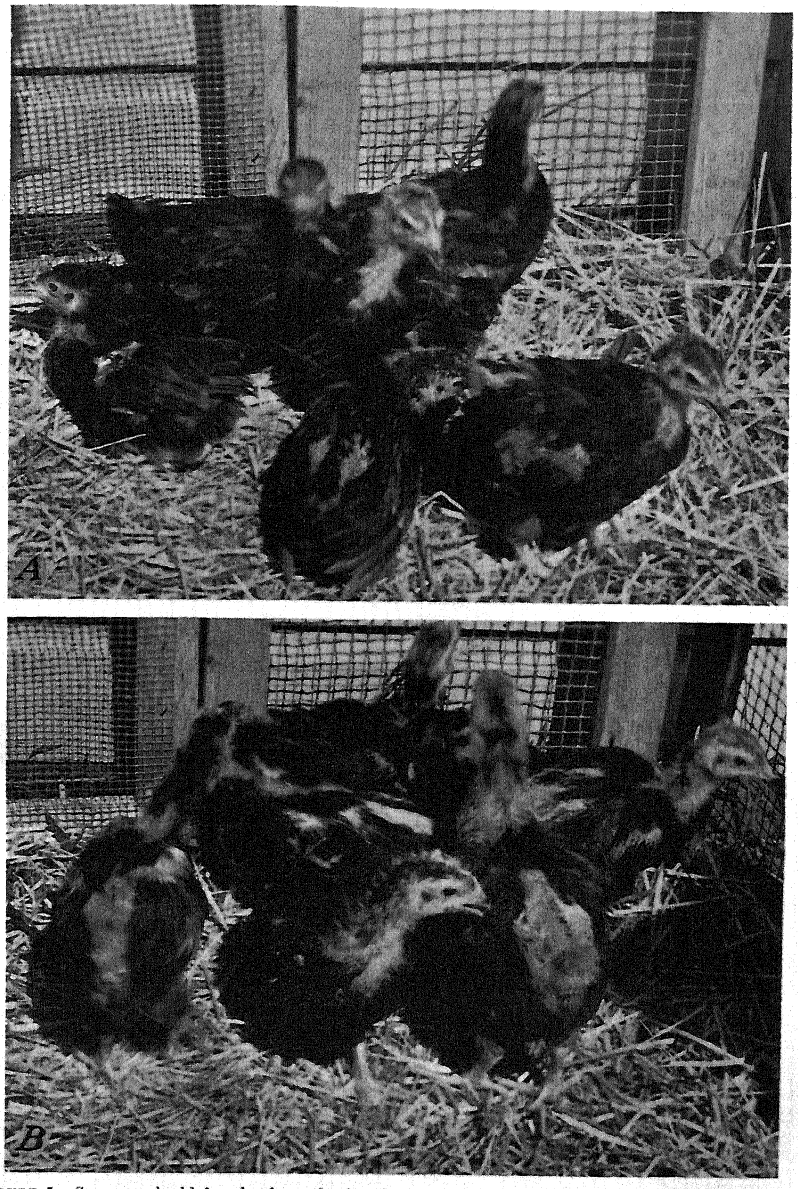


FIGURE 5.—Seven-week-old females from the fourth generation of selections of the well-feathered strain (*A*) and the poor-feathered strain (*B*).

scores made in the F_1 generation, together with those in the two parental strains for the same year, are shown in figure 6.

TABLE 10.—*Effects upon feathering score and body weights of reciprocal matings between well- and late-feathered Rhode Island Reds, 1935*

Mating	Males			Females		
	Birds	Mean feathering score	Mean body weight	Birds	Mean feathering score	Mean body weight
	Number		*Grams*	*Number*		*Grams*
Poor-feathered male and well-feathered female	43	8.1±0.25	345.3±9.31	51	13.8±0.36	338.0±6.17
Well-feathered male and poor-feathered female	61	8.8± .24	371.0± 9.15	53	13.1± .43	333.6± 7.04
Well-feathered strain	77	9.2± .25	312.5±16.61	54	16.0± .44	306.3±20.67
Poor-feathered strain	59	5.1± .18	330.0± 6.63	36	8.4± .40	316.9± 7.65

These results show the condition of well feathering as being incompletely dominant to poor feathering, since the mean feathering

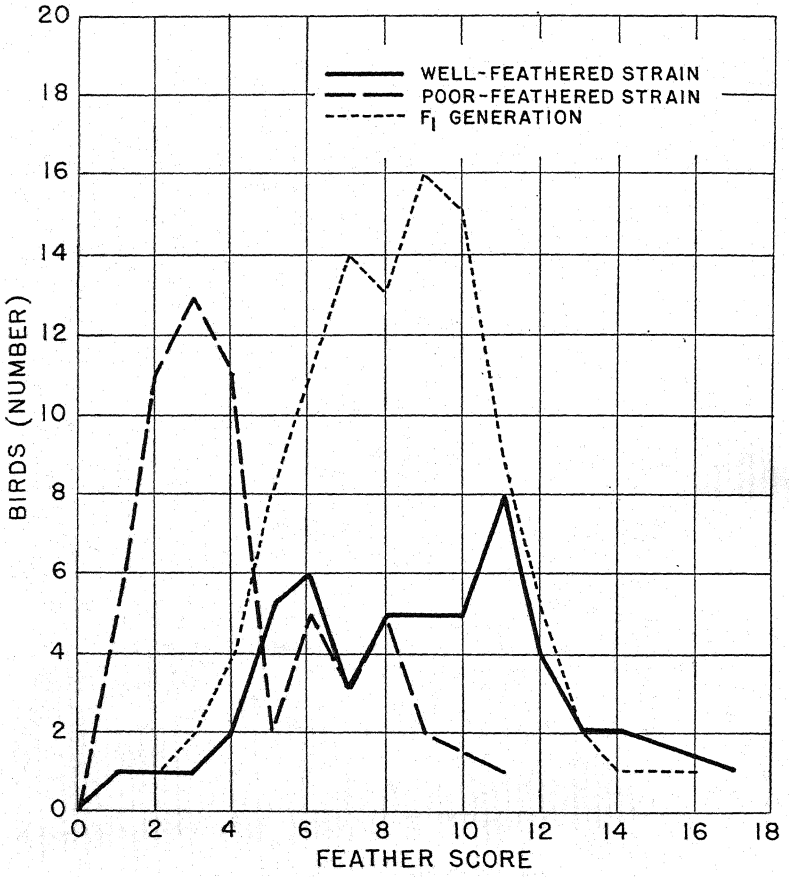


FIGURE 6.—Distribution curves for male feathering scores made in the well- and poor-feathered strains, together with that of the F_1 generation.

scores of the two sexes in the F_1 generation approximate very closely those of the well-feathered strain for the same year. The similarity in feathering score of the F_1 generation females from reciprocal crosses

shows that no important sex-linked factors are involved. Evidence is also adduced from these results that the progeny of these crosses have benefited by hybridization (*21*), as indicated by their mean body weight in comparison with that of the lines of selection in the same year. The growth of feathers did not seem to have been affected in the same manner. The feathering score of the hybrids was similar but slightly less than that of the well-feathered strain for the same year.

Backcrosses

F_1 generation females were backcrossed to males of both parent stocks. For this work females were chosen the feathering score of which was near the mean of the F_1 population. Approximately equal numbers of pullets from each of the reciprocal matings were placed in each pen. Again, in this generation the males used were mated with both F_1 generation females and those from their own strain, making possible comparisons between related pure strain and backcross progeny.

The results of the two backcross matings are summarized in table 11. The 168 birds produced by the matings headed by the well-feathered males had feathering scores of 8.8 and 13.1 points for the males and females, respectively. These two scores approximate very closely the means secured in the F_1 population and also the mean of the well-feathered selection line of 1936. The mean feathering scores of the progeny from the backcross to the poor-feathered parent were 5.7 and 8.7 points for the males and females, respectively. These scores are intermediate between the scores of the well-feathered and those of the poor-feathered strains, which again fits the theory of the dominant behavior of well feathering. Distribution curves of male feathering scores in the backcrosses to the parental stocks are shown in figure 7.

Table 11.—*Effects upon feathering score and body weights of backcross matings of F_1 generation females to the two parent stocks*

Mating	Males			Females		
	Birds	Mean feathering score	Mean body weight	Birds	Mean feathering score	Mean body weight
	Number		*Grams*	*Number*		*Grams*
Well-feathered male and F_1 females	45	8.8±0.27	340.7±4.78	124	13.1±0.25	327.6±4.76
Poor-feathered male and F_1 females	123	5.7± .15	355.6±5.60	150	8.7± .18	324.4±4.28
Well-feathered strain	50	9.0± .31	337.0±8.18	112	14.0± .24	325.2±4.26
Poor-feathered strain	58	4.4± .21	325.2±8.01	75	6.8± .25	300.3±5.84

The results obtained from these backcross matings seem to offer additional support to the view that the condition of being well-feathered is incompletely dominant to poor feathering. This is in agreement with the findings of Jaap and Morris (*9*). In dealing with a quantitative character of this type, it is difficult to determine exactly how many factors are involved, but the genetic behavior of the character is such as to suggest the action of relatively few major factors and that these are autosomal in location. The early establishment by selection of the differences secured would support the

RHODE ISLAND RED—WHITE LEGHORN CROSSES

Reciprocal crosses between poor-feathering Rhode Island reds and late-feathering White Leghorns were made to determine the extent to which poor feathering, as it exists in the Rhode Island Reds, differed from late feathering as it is carried by the Leghorns. As stated

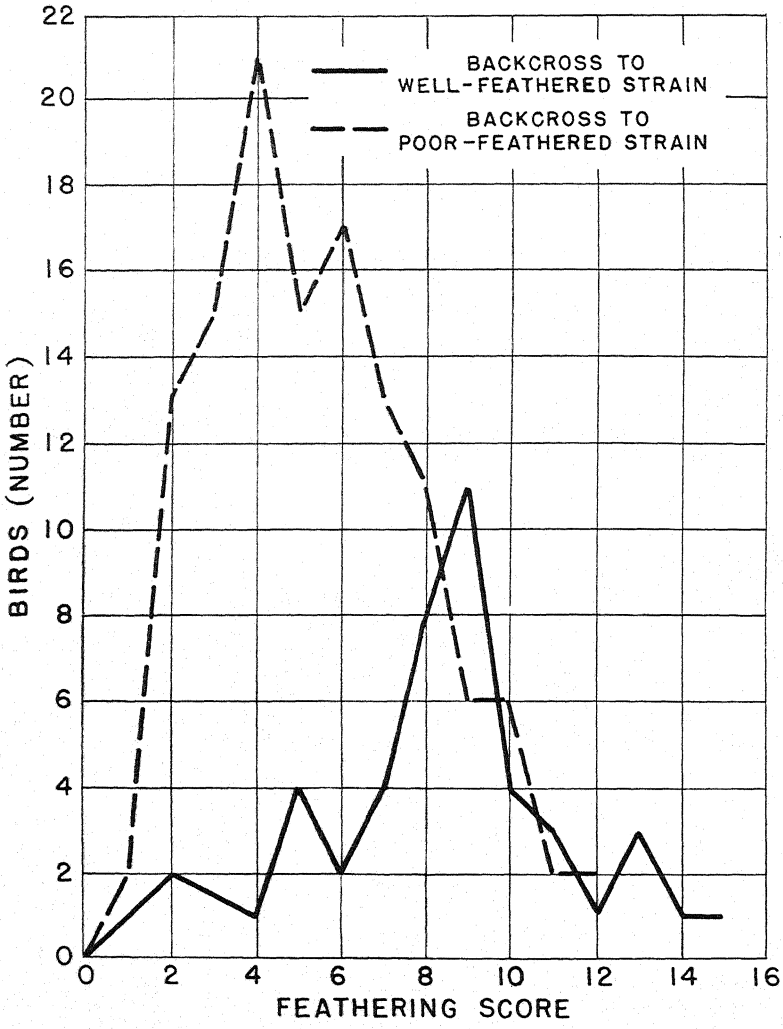


FIGURE 7.—Distribution curves for the male feathering scores in backcrosses to the parental stocks.

previously, the late-feathered Leghorn birds used were the result of selections from a cross of normal-feathered Leghorns and Barred Plymouth Rocks. The ultimate results of these selections were birds very similar to normal Leghorns except that they carried the sex-linked dominant gene for late feathering from the Barred Plymouth Rocks. A total of 129 birds was produced from the mating of the poor-feathered Rhode Island Red cock with late-feathering Leghorn

females. Of these offspring 79 were male and 50 female. The mean feathering score of the males was 8.6 points, while that of the females was 14.1. The reciprocal mating headed by the late-feathered Leghorn male produced 127 birds having a mean feathering score of 8.7 for males and 14.5 for females. The offspring of the reciprocal crosses had almost identical mean feathering scores, and these scores were very similar to those of the well-feathered Rhode Island Red and the F_1 generation of the cross of poor- by well-feathered Rhode Island Reds. Thus in crosses the slow-feathering Leghorn strain behaves genetically very similarly to the well-feathered Rhode Island Red strain. An examination of table 1 reveals the fact that these two strains differ somewhat in the time of feathering, the slow-feathering Leghorns growing feathers somewhat earlier. These differences would suggest that the slow-feathering Leghorns and well-feathered Rhode Island Reds differ genetically to a degree, but these differences are not expressed when the two strains are crossed to the poor-feathered Rhode Island Red strain.

It should be kept in mind that the variations considered in the genetic studies, were those occurring in the strains of Rhode Island Reds known to be homozygous for the dominant sex-linked late-feathering factor. Most strains of Rhode Island Reds carry the late-feathering factor as contrasted with the early-feathering found in Leghorns.

CONCLUSIONS

Well-feathering is incompletely dominant to poor-feathering and is conditioned by autosomal factors. In the case of the sex-linked pairs of genes influencing feathering, better feathering is the recessive. Although physiological relationship between these types of feathering seems evident, their hereditary behavior appears to be quite dissimilar.

DISCUSSION

It appears that the genetic method may offer a simpler approach to the problem of broiler feathering than the physiological one. Nutritional or other physiological methods offer some possibility of improvement of feathering, but they are not always practical or economical. Selection in ordinary late-feathering strains will accomplish rather promptly some improvement in feathering at the broiler stage. The results indicate, however, that a condition of feathering, such as is found in the sex-linked, early-feathering strain, cannot readily be attained by selection of the genetically late-feathering stock. Sex-linked, early feathering, commonly present in the breeds of the Mediterranean class, and occasionally found in individuals of the large breeds, probably offers the most practical solution of the problem. The detection and propagation of the sex-linked early feathering gene in breeds utilized for broiler production is probably the simplest solution for the problem of broiler feathering.

SUMMARY

Vitamin A and the choice of grains used in the ration were of questionable significance in broiler feathering.

Thyroxine injections definitely stimulated feather development.

Brooding chicks under either high humidity or low temperature improved the feathering.

Molt was not a factor influencing the results of the experiments reported.

Selection carried out for a period of 4 years was effective in establishing strains differing genetically in degree of feathering at 7 weeks of age. Most of the established differences were accomplished in the first 2 years of selection.

Better feathering as established by selection was incompletely dominant to poorer feathering. The number of genes involved was not determined but the results would indicate that they are relatively few.

The genetic differences established by selection for degree of broiler feathering were probably due to modifying factors acting upon the sex-linked dominant late-feathering factor.

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SORGHUM CHARACTERS GROUPED BY MULTIPLE CORRELATIONS[1]

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INTRODUCTION

The heritable characters of grain sorghums (*Sorghum vulgare* Pers.), which have been suggested as sources of linkage groups, were used for determining their practical value as a means of indicating yields. The approach has been from a multiple correlation basis. Thirteen such characters have been correlated with yield at various times during eight seasons. Ayyangar and others[2] who conducted a similar investigation over a much shorter period, have drawn the following conclusion: "The total grain yield of a plant can be predicted very closely, when the diameter of the peduncle, length and thickness of the ear head, and the weight of 100 grains are all known."

MATERIAL AND METHODS

A white-seeded grain sorghum, Blackhull, was crossed with an amber-seeded sorgo (sweet sorghum), Japonica, and the hybrid was used throughout the investigation. Blackhull is about two-thirds as high as Japonica; it is nonsaccharine, while Japonica is sweet; its heads are compact, while those of Japonica are loosely branched; and its leaf surface is much smaller than that of Japonica. Head-to-row plantings were made each year, and 10 plants were measured from each of 20 rows. Heads representing a medium between the contrasting characters were selected each year from the rows, thus maintaining a type similar to the F_1 of the original cross.

The 13 characters measured are listed below, together with the method of determining each. Nine independent variables were used each year to check against the yield of grain as the dependent variable. Those found to be of low scoring value were dropped and replaced by more promising ones.

(1) Lengths of seed branches—measured in tenths of an inch by means of a caliper.
(2) Length of plants—measured by a surveyor's rod in feet and tenths of a foot.
(3) Length of heads—measured upon the same rod in tenths of a foot.
(4) Circumference of heads—determined by calipering the greatest diameter.
(5) Length of tip branches—determined by a caliper in tenths of an inch.
(6) Circumference of stalks—estimated from the calipered diameter.
(7) Number of nodes in heads—counted only on plants that developed definite heads.
(8) Number of side branches—counted only on plants that developed definite heads.
(9) Length of the last internodes—measured in feet and tenths of a foot on the surveyor's rod as head and length of plant were determined.
(10) Number of leaves—counted.
(11) Leaf area—determined by means of a planimeter.

[1] Received for publication November 20, 1937; issued May 1938.
[2] Ayyangar, G. N. Rangaswami, Ayyar, M. A. S., Hariharan, P. V., and Rajabhooshanam, D. S. The relation of some plant characters to yield in sorghum. Indian Jour. Agr. Sci. 5: 75–100. 1935.

(12) Chlorophyll—determined by a photoelectric colorimeter reading of an alcoholic extract of ground leaf material. These readings are indicated in foot-candles of light passing through a measured amount of solution.

(13) Sugar content—measured in a few drops of freshly expressed juice by a Zeiss hand sugar refractometer. The value for sugar content is expressed as a percentage of the total solids present in the juice. Collections were made each Monday at 9 a. m., and the juice was expressed into the refractometer at once to prevent any decomposition of the sugar. This process was started as soon as the stalks began to show nodes and was continued until the end of the growing season. The percentages are averages of the entire series.

Nine variables were measured for each plant, 10 plants being used from each head-to-row planting. In the case of sugar and chlorophyll determinations, samples were taken each week during the entire growing season and the average was used in the correlations.

The tabulations were made according to the plan of Wallace and Snedecor [3], without classifying into groups. The steps were followed as suggested in their formulae for determining multiple correlation coefficients. Solutions of the beta values are expressed in a multiple correlation coefficient, with a standard error of estimate, for the years 1929–36, as shown in table 1.

TABLE 1.—*Coefficients of multiple correlation and standard errors of estimate, 1929–36*

Item	1929	1930	1931	1932	1933	1934	1935	1936
Coefficients.....percent..	74.2	89.0	76.2	96.2	8.3	77.4	39.5	58.7
Standard error of estimate.....	41.6	64.8	24.6	29.0	2.3	48.7	8.4	30.9

EXPERIMENTAL DATA

In the first 4 years the correlation percentages increased in a significant trend, while the standard error of estimate decreased, but during the last 4 years the results were irregular, with a notable decrease in 1933. Standard errors of estimate imply an unusual variation in the applicability of the data. In 1934, the error was very high as compared to the coefficient of that year, but it was not so divergent as that of 1930. Since it has been suggested that the moisture supply was responsible for these wide variations, the rainfall for the growing periods of the years involved is shown in table 2.

TABLE 2.—*Summer rainfall at the Oklahoma Agricultural Experiment Station, 1929–36*

Year	May	June	July	August	September	October	Total	Average
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
1929	8.29	2.11	2.66	.43	2.66	4.38	20.53	3.42
1930	6.23	2.36	.04	3.84	.91	2.02	15.40	2.57
1931	2.02	2.24	3.56	3.67	2.01	.99	14.49	2.41
1932	2.28	6.47	9.56	5.55	.94	3.71	28.51	4.75
1933	1.73	.00	6.81	4.99	1.44	4.15	19.12	3.18
1934	2.68	2.07	.72	3.19	8.95	2.23	19.84	3.30
1935	3.59	10.31	.51	3.08	2.26	2.18	21.93	3.65
1936	4.84	1.91	.37	.00	5.77	2.31	15.20	2.53

Rainfall during the early growing season of 1933 was very light, with a heavy July and August moisture supply. The result was an unusual secondary growth of side branches and small heads during

[3] WALLACE, H. A., and SNEDECOR, G. W., CORRELATION and MACHINE CALCULATION. Iowa State Col. Pub. 30, 71 pp., illus. 1931.

the latter part of the season. Since Japonica has a tendency to branch more than Blackhull under moist conditions, there are some marked inconsistencies in yield. During 1935 and 1936, the secondary growth occurred later and did not affect the yield as much as it had in 1933. The results in table 3, which shows the comparative scores for each year of the project, may contradict the above supposition. In 1933, the large-scoring factors were length of last node and leaf area, with a small value for number of side branches. Comparative scores are expressed in percentages of the dependent variable.

TABLE 3.—*Comparative scores, showing effect upon yield of each factor*

Factor	1929	1930	1931	1932	1933	1934	1935	1936
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Length of seed branches	41.60	3.0	13.99	6.95	8.0	10.35	1.28	7.57
Length of plant	1.93	15.9	18.05	7.47	6.5	13.42	8.23	0
Length of head	11.93	13.3	16.03	8.79	13.0	(1)	(1)	16.72
Length of tip branch	1.40	15.7	4.06	(1)	(1)	(1)	(1)	(1)
Circumference of head	24.44	26.4	24.52	39.09	.5	16.88	8.92	4.94
Circumference of stalk	0	0	0	(1)	(1)	(1)	(1)	(1)
Number of nodes in head	2.87	7.6	1.05	(1)	(1)	(1)	(1)	(1)
Number of side branches	0	11.7	15.59	1.36	2.0	23.0	7.82	9.37
Length of internode	15.63	6.2	6.41	1.29	37.0	6.67	16.15	11.17
Number of leaves	(1)	(1)	(1)	11.7	(1)	(1)	(1)	(1)
Leaf area	(1)	(1)	(1)	.40	22.0	14.60	37.54	12.54
Chlorophyll content	(1)	(1)	(1)	22.94	11.0	15.99	10.81	18.11
Sugar content	(1)	(1)	(1)	(1)	(1)	(1)	9.25	19.55
Total effect on yield	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.00

[1] Not determined during that year.

Since a large number of variables involves much greater complications and increases the probability of error, those of least significance were dropped. The scores of the last 3 years show that leaf area, chlorophyll, and sugar content formed a large portion of the contributions to yield. The first three lengths measured from a group of comparable value, which Karper and Quinby[4] consider as coming from closely related genes. Leaf area, sugar content, and chlorophyll form a second comparable group, and number of leaves and nodes a third.

TABLE 4.—*Solution of normal equations; simple correlation coefficients, 1935–36*

Variables	Correlation coefficients [1] in class—									
	1	2	3	4	5	6	7	8	9	10
1. Length of seed branches	1.000	+0.432	+0.401	−0.168	−0.242	+0.933	−0.189	−0.019	+0.884	+0.051
	1.000	−.048	+.083	−.138	−.549	+.218	+.056	+.441	−.098	+.468
2. Length of plant	------	1.000	+.161	−.071	−.236	+.079	+.316	+.277	+.217	−.179
	------	1.000	+.149	+.188	+.208	+.155	+.181	−.275	+.419	−.186
3. Leaf area	------	------	1.000	−.181	−.203	+.384	−.084	−.059	+.285	+.385
	------	------	1.000	−.161	−.004	+.233	−.657	−.675	+.082	−.249
4. Chlorophyll content	------	------	------	1.000	+.330	−.028	+.437	+.030	−.134	−.163
	------	------	------	1.000	−.510	+.379	−.067	+.171	+.039	+.008
5. Sugar content	------	------	------	------	1.000	−.201	+.558	+.154	−.216	−.099
	------	------	------	------	1.000	−.207	−.218	−.378	−.195	−.296
6. Circumference of head	------	------	------	------	------	1.000	−.252	+.089	+.779	+.043
	------	------	------	------	------	1.000	−.218	−.029	−.025	−.296
7. Length of last node	------	------	------	------	------	------	1.000	+.142	−.185	−.279
	------	------	------	------	------	------	1.000	−.221	+.705	+.300
8. Number of side branches	------	------	------	------	------	------	------	1.000	−.276	−.077
	------	------	------	------	------	------	------	1.000	−.474	+.165
9. Length of head	------	------	------	------	------	------	------	------	1.000	−.083
	------	------	------	------	------	------	------	------	1.000	+.224
10. Yield	------	------	------	------	------	------	------	------	------	1.000
	------	------	------	------	------	------	------	------	------	1.000

[1] Second line of data shows in each case the 1936 correlation coefficients for comparison with 1935, given first.

[4] KARPER, ROBERT EARL, and QUINBY, J. R. HYBRID VIGOR IN SORGHUM. Jour. Hereolity 28: 83-91, illus. 1937.

Table 4 shows that the comparative value of individual determinations for the respective years is slight.

A comparison of sugar content (5) with circumference of head (6) for 2 years shows a negative correlation for both years. In 1935, the same sugar content had a positive correlation with length of last node of 0.588, indicating some significance, but in 1936 it had a negative correlation of 0.218. The negative correlation of sugar content with yield of grain is to be expected, because the so-called high-yielding grain sorghums are seldom sweet-stalked. Leaf area (3) shows a positive correlation of 0.385 with yield in 1935, but in 1936 there was an insignificant negative correlation of 0.249.

TABLE 5.—*Solution of beta values for various factors, 1935–36*

Year	Length of seed branches	Length of plant	Leaf area	Chlorophyll content	Sugar content	Circumference of head	Length of last node	Number of side branches	Length of head
1935	−0.8111	−0.0004	+0.4578	−0.0950	−0.0625	+0.0992	−0.1714	+0.0307	+0.5926
1936	−.1294	−.5637	+.1555	+.4771	−.5574	−.2806	+.0772	−.0268	+.3950

A comparison of values in table 5 confirms the inconsistencies of the correlations shown in table 4. The last pair of values for head lengths may suggest a trend of some significance, but the others have such a wide spread from positive to negative that comment is not necessary. Because of the limited number of samples, there may be some doubt as to the applicability of these measurable characters as indicators of yield.

SUMMARY

Whether yields can be accurately predicted from the several score values indicated in these studies is doubtful, especially in seasons of subnormal rainfall.

The irregular results obtained in 4 of the 8 years show that moisture and temperature have much to do with the correlation values.

A basis for inheritance studies may be found in three groups of values shown in this paper: (1) length of plant and plant parts (head and seed branches); (2) photosynthetic agencies, such as leaf area, chlorophyll, and sugar content, and (3) number of leaves and nodes.

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DIRECTIONAL PERMEABILITY OF SEASONED WOODS TO WATER AND SOME FACTORS WHICH AFFECT IT[1]

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INTRODUCTION

The study of wood permeability is a comparatively recent innovation in the field of scientific research. Investigations on plants elucidated the finer structure of woody stems, which in turn has led to speculation on the probable mechanism of liquid movement in wood.

The earlier investigations on fluid penetration into wood were made by those who were interested in the preservative treatment of wood. Subsequent research has been conducted on a much broader basis and has employed various methods of attack of a physical and chemical nature in an attempt to arrive at fundamental principles of wood permeability as well as improvements in various industrial aspects of the treatment of wood. One conclusion which stands out from a survey of the literature and from work in the field, is that the flow of liquids through wood is a complicated problem influenced by numerous factors, some of which are inherent in the structure of wood while others arise from the relationship of wood to the properties of a liquid.

Previous investigations by the authors represented an attempt to clarify certain controversial points concerning the effect of various treatments and liquids on wood permeability and to obtain a more complete knowledge of the comparative longitudinal permeability of woods.

Very little work has been done on the measurement of the rate of liquid flow through wood radially and tangentially, although information on the relative penetrance from impregnation studies has been reported. Since such permeability, or the lack of it, is important in many commercial uses of wood and in processes involving contact and penetration of liquids, information to aid in a fuller understanding of the subject seemed desirable. Accordingly a study of radial and tangential permeability of a number of woods was made with attention being given also to the comparative permeability of springwood and summerwood and of heartwood and sapwood in the two directions where possible.

REVIEW OF LITERATURE

In order to explain the penetration of preservative fluids into wood, Tiemann (*27*)[2] advanced the hypothesis that when wood was seasoned below the fiber-saturation point, narrow microscopical slits developed in the walls of the fibers and tracheids which rendered them penetrable to liquids. In common with the botanists of his time, he believed that the cells of wood in the green state were enclosed by a continuous

[1] Received for publication June 28, 1937; issued June 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 744.

primary wall through which liquid might pass but only very slowly even at high pressure. The difficulty of injecting air and preservative oils seemed to substantiate this.

Weiss (*28*) observed that there was greater penetration of creosote in the summerwood than in the springwood of the same annual ring in longleaf pine. This he explained by saying that the thick walls of the summerwood would crack more on drying than the thinner walls of the springwood.

On examination of creosoted blocks, Bailey (*1*) found that in most cases the secondary walls of the summerwood cells were unruptured and that even when ruptures occurred the primary walls remained intact. He also noted that air passed very readily through the dry springwood in which the walls were not checked. He observed (*2*) that carbon particles in suspension passed through unseasoned sapwood. From microscopic and photomicrographic studies he concluded that the pit membranes were not entire but had minute perforations in the thinner radii of the membrane. In studies on mercury penetration, Scarth (*16*) found that the mercury apparently passed to the adjoining tracheids through the pit membranes when the torus was in the normal, median position. When pits were absent or closed (aspirated) the mercury did not enter the cells.

From the results of penetration of a mercury sol and india ink into wood, Stamm (*18*) decided that if openings other than those in the pit membranes existed, they were too small to have an appreciable effect on the determination of membrane pore sizes. He found further that when the length of the effective capillaries between tracheids was taken as the thickness of the pit membrane (assumed to be approximately that of the middle lamella) in calculations to determine the size of the effective openings by electroendosmosis, the values approximated those obtained by hydrostatic methods. Very different values were found if the length of the capillaries was assumed to be the thickness of the cell wall. In a recent review, Stamm (*21*) states that the cell-wall permeability under pressure is negligible as compared to the pit-membrane permeability except in extremely resistant woods.

Much of the information on the effective capillary dimensions has been determined by Stamm. The methods which he has employed include electroendosmotic flow, hydrostatic flow, gaseous flow, overcoming surface tension in the capillaries, electrical conductivity, and permeability to colloidal sols.

The effective capillary cross section of some softwoods cut in the three structural dimensions was determined by electroendosmosis (*18*). By measuring the electrical conductivity of salt solutions in wood the ratio of the effective capillary length to the effective continuous capillary cross section was determined (*19*). By combining the data of the first method with that obtained by hydrostatic flow (pressure-drop method) the average effective diameter of the openings (calculated as circles) for the heartwood of two conifers was found to be from 11 to 23 mμ. The range in maximum effective diameters of five species was from 68 to 184 mμ (*18*). The average values obtained by a similar combination with electrical conductivity data (*19*) were later found to be in error owing to an invalid assumption. The maximum values were determined in all experiments by calculation from the pressure necessary to overcome the surface tension of the

water in the largest effective capillary. In the latter case (*19*) the maximum effective capillary radii were from 40 to 120 mμ for the heartwoods of several species and from 180 to 11,000 mμ for the sapwoods. Stamm (*20*) has suggested a method for determining the distribution of the size of openings in a membrane by the rate of change of the velocity of flow of air with changes in the effective capillary radius which varies with moisture content or relative humidity. The minimum effective capillary radius was determined by the departure from a linear relationship between moisture content and permeability to air at the appropriate relative humidity. The minimum effective radius for a tangential section of white pine heartwood was 10 mμ, the average radius 27 mμ, and the maximum radius 73 mμ.

The probable importance of aspirated pits on the penetration properties of wood has been emphasized by several investigators. Griffin (*7*), one of the earliest workers on this problem, determined by actual count the proportion of unaspirated and aspirated pits in seasoned and unseasoned Douglas fir for both the mountain and lowland types. The latter had relatively few aspirated pits in the green state as compared to the former. Air drying caused an increased number of aspirated pits in both the springwood and summerwood of the mountain type, whereas in the lowland type this occurred only in the springwood. Soaking the heartwood in alcohol before drying it prevented pitted aspiration.

Griffin (*7, 8*) obtained a definite relation between creosote penetration in the wood and the proportion of unaspirated pits. Scarth (*16*) reported similar results on mercury penetration. MacLean (*13*) found a considerable proportion of aspirated pits in several woods, the springwood having a greater proportion of closed pits than the summerwood. He also believed this to be "at least partly responsible" for the difficulty of treating such woods.

Phillips (*15*) recently has studied the extent and time of occurrence of pit aspiration in five species of softwoods. The general conclusions already given were verified except that drying below the fiber-saturation point did not increase the number of aspirated pits. Pit aspiration increased gradually during the drying of green wood until nearly at the fiber-saturation point when almost all of the springwood pits became aspirated. The abrupt change indicated that aspiration was associated with the loss of the last traces of free water in the cells. The proportion of unaspirated pits was found to increase with increasing thickness of the cell wall when the diameter of the pits in the summerwood remained nearly the same. The average number of unaspirated pits per tracheid were from 0.5 to 6.3 for the springwood and from 2 to 11 for the summerwood in the air-dry wood.

Scarth and Spier (*17*) suggest that pit membranes are pulled over to the pit aperture because of resins and other colloidal material released into the tracheids at the time of the death of the living cells. The wood then becomes heartwood in character. Nonresinous conifers showed pit aspiration to a lesser degree, which also explained the greater permeability of their heartwood. Phillips (*15*) states that closure of pits may be caused by evaporation of the last drop of water in the pit cavity, causing the torus to be drawn gradually toward the pit aperture by the surface tension of the annular meniscus between the pit border and the torus. This may be true for drying

just above the fiber-saturation point, but it is doubtful whether it explains heartwood formation, for heartwood is not often at or below the fiber-saturation point.

Bailey (*2*) and Scarth (*16*) believe that the application of pressure probably forces the torus against the pit aperture, thus exerting a valvelike action and preventing the flow of liquid unless (*16*) the pressure is sufficient to rupture the pit membranes. Sutherland, Johnston, and Maass (*23*) and Buckman, Schmitz, and Gortner (*5*) found that permeability continued to increase with an increase in pressure with no sign of tapering off or decreasing as higher pressures were applied. Hence no valve action of the tori could have occurred. That the membranes had not been ruptured by pressure was shown by the fact that approximately the same curve was traversed by the stepwise reduction of pressure if adequate time was allowed for the attainment of an equilibrium rate of flow.

The comparative permeability of sapwood and heartwood is apparently characterized by a great variation for different species. No great amount of work has been done with specific intent to settle the question, but several reports give an approximate idea of what may be expected.

From tests on the relative ease of creosote impregnation of a number of conifers, Teesdale (*24*) found that the sapwood was more easily penetrated than the heartwood in those species possessing a highly developed system of resin canals. Species without resin canals were impregnated but little better in the sapwood than in the heartwood except in some woods with distinct color differences such as cedars, cypresses, etc. From impregnation studies on 25 hardwoods, Teesdale and MacLean (*25*) concluded that sapwood of all the species was fairly easy to penetrate radially even though the heartwood was resistant. These results are in agreement with the findings of Scarth (*16*) and Weiss (*28*). These qualitative results show the general trend and are the gist of information on lateral penetration. Sutherland, Johnston, and Maass (*23*) in only three comparative runs on seasoned white spruce, found that lateral rate of flow through the sapwood was about 10 times that through the heartwood. Their data are too meager, however, to permit any general conclusions to be drawn.

There is more information on the comparative longitudinal permeability of heartwood and sapwood. Johnston and Maass (*12*) found that longitudinal flow in sapwood of seasoned white spruce and green jack pine was 100 times faster than in the corresponding heartwood. Sutherland and others (*23*) reported that sapwood of three species was from 60 to 10,000 (calculated) times more permeable longitudinally than the corresponding heartwood.

The comparative heartwood and sapwood longitudinal permeabilities were determined directly at low pressure on three species of wood by Erickson, Schmitz, and Gortner (*6*). A wide range of ratios (from 10 to 500 times as permeable in the sapwood as in the heartwood) was found which was determined by the species, the treatment received, and the liquid used. Since the heartwood of the other species tested was not permeable at low pressure, the ratio in those species probably would be very much greater even at higher pressures.

Teesdale (*24*) noted that the color line between sapwood and heartwood of pines did not always coincide with the ease of permeability even in the same cross section of the tree. Scarth (*16*) likewise found

bands or areas of different resistance which extended radially or tangentially in the region of the border line.

The most important structural factor influencing penetration into hardwoods was found by Teesdale and MacLean (*25*) to be the relative absence of tyloses in the vessels. (This is undoubtedly of greatest importance in longitudinal penetration). Next in importance was the permeability of the wood cells to the fluid.

It is not yet clear what role resin canals play in wood permeability and impregnation of woods with fluids. Tiemann (*27*) found that resin canals may or may not permit air to pass through freely. He attributed the difference to the fact that some canals were open whereas others were clogged with resin. Weiss (*28*) explained the difficulty of treating Douglas fir and spruce by the relatively few resin canals which they possess; in southern pines resin canals were more abundant and the woods were easier to treat. Teesdale (*24*) also believed that the resin canal system acted as channels of flow for the preservative fluid. Radial resin canals were believed to be especially effective because when they were present radial penetration was from one-fourth to three-fourths of the longitudinal penetration. The response of one species to treatment could not be applied to another species of similar structure. Inherent differences between species must be recognized.

Weiss (*28*) believed that in Douglas fir heartwood the resin became hard and added to the difficulty of impregnation. Teesdale (*24*) extended this idea of harder and more insoluble resin to include heartwoods in general. He stated that the ease of penetration of the sapwood of pines, spruces, etc., seemed to be a result of the combined effect of the nonresinous structure and the resin canals. The sapwood of species containing canals showed the most erratic absorption, and the most resistant species gave the most uniform results.

In jack pine sapwood, Scarth (*16*) found that resin canals were the chief means of longitudinal penetration, but in the heartwood they were usually blocked by resin or by tylosoids. Johnston and Maass (*12*), after several methods of experimentation, did not deny the effectiveness of resin canals in jack pine heartwood, but their results demonstrated, they believed, that only a part of the flow occurred in this way and that cell penetration did take place under pressure. In attempts to penetrate the wood with safranine solution, they found the dye confined to the first tracheid length and to the resin ducts, but after one-half hour the whole sample was evenly saturated with water, and in spite of the failure of the safranine to move into the tracheids, they believed that this indicated that water moved through the section by means other than the resin ducts. In the opinion of Sutherland and others (*24*), longitudinal flow through sapwood was too rapid to be accounted for by resin canals.

No significant difference in longitudinal rate of flow was found in the areas with or without resin canals in the same part of the annual ring in two southern pine sapwoods investigated by Erickson, Schmitz, and Gortner (*6*).

Most of the available information on directional permeability has been obtained from impregnation studies on wood, although a few measurements of radial and tangential rates of flow have been made. Weiss (*28*) determined the creosote penetration in the three structural directions of several woods by forcing creosote into holes bored into

a wood block of given dimensions and then measuring the distance of penetration. Radial and tangential penetrations were less than the longitudinal penetration; the ratio between them varied with the species.

Teesdale (*24*) found that in woods with resin canals the radial penetration of creosote was from one-fourth to three-fourths of the longitudinal penetration and that the tangential penetration could be disregarded since it was usually a fraction of the radial value. In longleaf pine, for example, the longitudinal, radial, and tangential penetrations were in the ratio of 100 to 4 to 1. In woods without resin canals the radial and tangential penetration were about equal and only one-twentieth to one one-hundredth of that obtained longitudinally.

Johnston and Maass (*12*) reported about the same magnitude of flow in the three directions for jack pine sapwood. In the heartwood, however, longitudinal flow was 100 times that in the two lateral directions. Sutherland and others (*23*) found that in the heartwood of white spruce the apparent penetration radially and tangentially was less than 9 percent of the longitudinal value, although the actual amount penetrating was about 1 percent. In sapwood the radial penetration was less than 2 percent of the longitudinal value. In unseasoned Norway pine sapwood the radial and tangential penetration was less than 1 percent of the longitudinal value. Tangential penetration of white spruce sapwood was somewhat greater than radial penetration. Sutherland and his coworkers admit, however, that these results are not satisfactory largely because of experimental difficulties.

Stamm (*20*) has used radial and tangential permeability to some extent in determining the effect of moisture content on the effective size of the capillaries in the pit membrane and to find the effective size of the capillary openings (*18*).

It is obvious that suitable and extensive data do not exist on directional permeability.

The suggestion is found occasionally in the literature that possibly wood rays aid in the radial movement of liquids in wood. However, relatively few observations have been made and very little study has been done on this specific problem. Teesdale and MacLean (*25*) observed little or no creosote in the wood rays or other parenchyma cells of hardwoods after the preservative treatment. In conifers, likewise, Teesdale (*24*) found that as a rule creosote was not present in wood rays after pressure treatment. In Bailey's (*2*) experiment, carbon particles of the size used did not enter the wood rays. However, Stamm (*18*) pointed out that the effective open capillary structure of tangential sections determined by electroendosmosis corresponded to that of the internal cross-sectional area of the ray cells obtained by microscopic measurements.

It is evident from the above statements that the role of wood rays in the radial permeability of wood is undetermined and that more proof is needed before either view can be accepted. The weight of evidence seemingly favors the idea of impermeability of the wood rays.

There is scattered information in the literature relative to the comparative permeability of springwood and summerwood. In dry loblolly pine sapwood Tiemann (*27*) observed relatively greater permeability in the springwood to air passing in the longitudinal direction by his method of soap films on the surface of the wood.

Weiss (*28*) and Bailey (*1*) observed greater absorption of creosote in the summerwood than in the springwood of longleaf pine. From rather extensive experiments with creosote injection into conifers, Teesdale (*24*) decided that in most woods summerwood was more penetrable than springwood. Redwood, yew, and tamarack were exceptions. Depth of color was used as a basis of comparison. An analysis of one wood, loblolly pine, showed that the summerwood contained 80 percent more creosote than the springwood.

In later work MacLean (*13*, *14*), Teesdale and MacLean (*26*), Griffin (*8*), and Scarth (*16*) found greater penetration and absorption of the preservative fluid in the summerwood by several species of softwoods, principally longleaf pine, Douglas fir, corkbark fir, and spruce. In some species it was often difficult to determine which was the more permeable to zinc chloride (*13*), but with creosote the summerwood was usually penetrated most readily. Scarth and Spier (*17*) forced water-soluble dyes into red spruce sapwood longitudinally and noted quick staining of the springwood but frequently no staining of the summerwood. In the heartwood, contrary results were obtained. Buckman (*4*) found that in the sapwood of freshly creosoted southern yellow pine, the springwood of the majority of the rings contained more creosote than the summerwood. The greater void volume of the springwood afforded a probable explanation. The higher creosote concentration in the summerwood of some rings was attributed to a greater penetration. This occurred only when the total absorption of the ring was low or when conditions of relative free space differed prior to treatment. Buckman pointed out that the data probably could not be justifiably interpreted as meaning a generally greater permeability in the summerwood, although the evidence favored it.

Erickson, Schmitz, and Gortner (*6*) measured the rate of longitudinal flow through the springwood and summerwood of the sapwood of two southern yellow pines to obtain the desired comparison. In both species the springwood was very definitely more permeable to water than the summerwood in both the seasoned and unseasoned specimens. This apparently contradicted most of the preceding work on the problem, but the authors pointed out that the vastly different conditions of the two types of experiments might possibly account to some extent for the disagreement in results on the same woods as reported by others.

Bailey (*2*), after postulating the existence of openings in the pit membrane, explained the great resistance of green woods to flow of air as being due in part to the effect of surface tension in the small openings where numerous membranes had to be traversed. Howald (*10*) could find no constant relationship between the surface tension at an oil-air or an oil-water interface and the penetration of the oil. This does not necessarily exclude the effects of surface tension but probably means that moderately small differences in absolute values for the different oils do not give apparent differences in their penetrance into wood.

According to Scarth (*16*), the sapwood of spruce resisted the penetration of mercury, which stopped at the pits. However, the hindrance was not a structural one, for water flowed through the sapwood and through the pits, as was shown by coloring the liquid. Hawley (*9*) points out that if a liquid does not wet wood the capillary forces

act in the opposite direction of water or against the movement into the capillaries. In such a situation a pressure as high as 50 atmospheres may be required to overcome the capillary forces in the pit membranes and cause intercellular flow. Although equally high capillary pressures may exist in the case of water, Hawley believes that they have little effect in aiding the capillary flow into wood and may actually be retarded by the pit membranes because of the large angle of their surfaces to the mouths of the openings.

Experimental evidence that the presence of air in the wood and that forces of surface tension may affect the rate of flow was obtained by Erickson, Schmitz, and Gortner (*6*). In a heartwood of moderate permeability the rate of flow of water initially and for a short time thereafter was usually less in the unevacuated sections than in the evacuated sections although the final rate of flow was about the same. In the more resistant woods, however, both the initial and the final rates were less in the unevacuated sections than in evacuated sections.

Sutherland, Johnston, and Maass (*23*) determined the effect of surface tension by forcing air through transverse sections of sapwood and heartwood. Difficulty in obtaining flow was encountered only in heartwood sections which had been partly soaked with water. They believed that the Jamin tube effect was responsible for the observed large increase in resistance with increased thickness of the wood section.

APPARATUS AND METHODS

The apparatus and materials used in determining the radial and tangential permeability of woods were essentially the same as those used by the present writers (*6*) in previous investigations on wood permeability.

The apparatus (fig. 1) was constructed entirely of brass in the parts subjected to pressure and of glass and rubber tubing in other parts. The essential features included a brass cylindrical supply tank (*b*) which contained the liquid and was capable of withstanding high pressure. This was connected to a feed line on which were four T connections at regular intervals. To these were attached brass steam valves, and in each was screwed the short length of pipe extending from the center of the lower brass disk. The pair of disks (*c*) was held in alinement by two pegs which fitted into holes in the other disk. The open area through the center of the disks or the area of the sections exposed to the liquid under pressure was 1.47 cm^2. To the top of each upper disk was sealed a glass T tube. On the side was attached rubber tubing and pinchcock and these served as a drain line for the liquid accumulated during the experiment. A burette (*d*) to measure the volume of liquid flow was fastened to the end of the T tube. In the runs in which the rate of flow was not rapid, which was the usual thing, pipettes were used instead of the regular size burette. These pipettes were calibrated to 0.01 cc.

A tank of compressed air (*a*) equipped with a reduction valve was used as a means of producing the desired hydrostatic pressure. The high pressures were read from a gage of 150 pounds per square inch maximum capacity; for low pressure a mercury manometer was employed. The time necessary for a given volume of liquid to flow through the section of wood was timed with a stop watch. In some preliminary determinations, it was found that at high pressure the

sections of some of the less dense woods bulged considerably in the open area exposed to the liquid. A brass retainer was inserted into the opening of the upper disk to serve as a backstop for the section if bulging should occur. This device was essentially a brass tube across one end of which were three raised bars with edges rounded to prevent the wood from being cut. The bars were spaced about 2 mm apart on either side of the center one. By the use of shims on the opposite end, the retainer was adjusted until the bars were only a few tenths of a millimeter beneath the surface of the disk. This seemed to eliminate quite satisfactorily the objectionable bulging and distortion.

No correction for the area of the bars has been made in data reported in this paper, for the following reasons: (1) It could not be definitely determined even on the same section from a number of tests whether

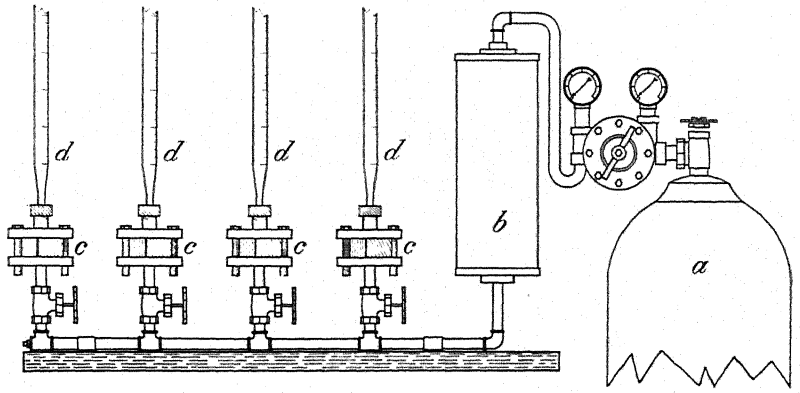


FIGURE 1.—Diagram of the apparatus showing the essential features. See text for description and explanation of lettering.

the retainer really did cause a decrease in rate of flow when the section was pressed against the bars. (2) In the woods tested at low pressure the section would not contact the bars firmly. (3) Not all sections would press against the bars with the same firmness even at high pressure, because the density and rigidity of the woods are variable. (4) It does not necessarily follow that contact with the section decreases appreciably the total flow in the region of the contact.

The initial rate of flow represents the volume of flow in cubic centimeters per minute usually in the first 2 or 3 minutes after the application of pressure. Where the flow of liquid was very slow more time was necessary to make finer adjustments before timing it. In very resistant sections the slight bulging of the section until firmly in contact with the retainer occurred in the first few minutes and might be mistaken for flow of liquid through the section. With some woods the rate of flow was so slow that 5 to 10 minutes was required to obtain a sufficient flow of liquid to permit an accurate measurement.

Most of the determinations were made at a temperature of about 24° C. When the rate of flow was exceedingly small, fluctuations in room temperature were held as constant as possible (within 1°); otherwise expansion and contraction of the liquids in the burettes or of the glass and other parts affected the smoothness of the readings to a noticeable extent.

The rate-of-flow values are nearly always given to three decimal places of significant numbers but not to more than five decimal places. The third digit and the fifth place were not always significant as determined by calculating the effect of probable errors in reading the pipettes and errors of calibration and long timing periods, but for the sake of uniformity these standards were adopted.

The two pressures used in these studies were 10 cm of mercury and 100 pounds per square inch gage reading. The lower pressure was used for those woods that were permeable to the extent of an observed flow of more than 0.002 cc per minute. After the regular 3-hour period of continuous flow, the pressure was increased in some runs to the higher pressure and the rate of flow was measured during a 3-hour period at this pressure. This was done for the woods shown in table 1 except white spruce sapwood, tamarack sapwood, and the sapwoods of loblolly pine and longleaf pine. The 3-hour period was selected as an arbitrary time limit because the rate of flow usually does not change rapidly with time after this period of continuous flow and because it facilitates comparisons on a common time basis. The term "final rate of flow" refers to the rate of flow after 3 hours of continuous flow.

The tangential sections of most species of wood were sawed from freshly felled trees of known history. The radial and tangential sections of eastern hemlock, western hemlock, northern white cedar, Douglas fir (coast type), and white oak were sawed from small seasoned blocks of the same logs used in the study of longitudinal permeability in previous work (*6*). The other radial sections were cut from small seasoned blocks of the same logs from which the tangential sections were sawed. The sections and blocks in the green condition were slowly dried in a cabinet equipped with a fan, two type C Bahnson humidifiers, heating elements, wet- and dry-bulb thermometers, and automatic controls for maintaining humidity and temperature. The temperature in the cabinet was 30° C. The woods were seasoned to about 8-percent moisture content. Sixteen species of wood were used in this investigation. The common and scientific names as given by Sudworth (*22*) are as follows:

Common name	*Scientific name*
"Resinous woods":	
Jack pine	*Pinus banksiana* Lambert.
Loblolly pine	*Pinus taeda* Linnaeus.
Longleaf pine	*Pinus palustris* Miller.
Norway pine	*Pinus resinosa* Solander.
Shortleaf pine	*Pinus echinata* Miller.
Slash pine	*Pinus caribaea* Morlet.
Tamarack	*Larix laricina* (DuRoi) Koch.
White spruce	*Picea glauca* (Moench) Voss.
Douglas fir (coast type)	*Pseudotsuga taxifolia* (Lamarck) Britton.
Black spruce	*Picea mariana* (Miller) Britton, Sterns, and Poggenberg.
"Nonresinous woods":	
Eastern hemlock	*Tsuga canadensis* (Linnaeus) Carrière.
Western hemlock	*Tsuga heterophylla* (Rafinesque) Sargent.
Balsam fir	*Abies balsamea* (Linnaeus) Miller.
Northern white cedar	*Thuja occidentalis* Linnaeus.
Paper birch	*Betula papyrifera* Marshall.
White oak	*Quercus alba* Linnaeus.

In each species the sections used were sawed from at least two different places in the log. Great care was exercised in cutting the

sections so that they would be as nearly perfect as possible. The trueness of the tangential sections to the longitudinal plane was judged by the resin canals and by the annual rings, and in the radial sections by resin canals or vessels when present, but most satisfactorily by examining the sides of the section through a hand lens and observing the deviation, if any, of the cell axis from the plane of the section. The trueness of the sections in the radial and tangential directions was judged by the direction of the wood rays as seen with the aid of a hand lens, and the direction of the annual rings, respectively. If the wood rays, starting near one corner, ran across the section to the other surface before the opposite side of the section was reached, the section was not considered acceptable. In all woods only the best of a number of acceptable sections was used. With some woods good sections were difficult to obtain.

To prevent the liquid that entered the section from flowing eut in a longitudinal direction particularly, but also from flowing out the sides, the ends and sides of the sections were impregnated with paraffin as thoroughly as possible by repeatedly dipping these parts in melted paraffin at from 70° to 75° C. Sudan III was used to color the paraffin, thereby aiding in observing the extent of penetration and capillary rise on the surface of the section while treating. In tangential sections, the resistance of the wood tangentially, which is equal to or greater than that radially, and the greater distance the water would have to move sidewise than through the section, would tend to be effective sealing agents in themselves by acting as hindrances to flow through the sides of the section.

In radial sections the argument of greater distance from the exposed area to the edge of the section also holds, but the resistance of the wood to flow would not be so great. However, in those woods that were very permeable radially, if leaking had occurred it should have been sufficient to be detected by dripping from the disks. No appreciable loss was noticed. The results on several woods, most of which were very resistant tangentially, were checked by applying several coats of a plasticized lacquer to other sections on all but the approximate area of the openings in the disks. The rate of flow or the absence of flow was about the same as in the paraffin-coated sections. Consequently, absence of flow or a very slow rate of flow was not due to leakage from the section. The woods which were checked were tangential sections of longleaf pine heartwood, shortleaf pine sapwood, white spruce heartwood, balsam fir heartwood, Douglas fir sapwood and heartwood, and tangential sections of northern white cedar heartwood. The lacquer method is probably the surest, but it is the most time consuming. The temperature of the paraffin probably caused no permeability changes in the wood, for the air above the liquid was not over 50° C., and the portion of the section not in the liquid would be less than 70° at least. Temperatures below this even on wet wood do not seem to affect permeability permanently (*23*).

All sections were humidified in a desiccator at 90 percent relative humidity prior to coating and also afterward over water for a total of at least 6 hours, and often much longer to prevent checking on the ends when increasing the moisture content after the coating process and before evacuation of the section.

Before each run the sections were evacuated 15 to 20 minutes over water and 10 to 15 minutes in water without breaking the vacuum,

the total time being about 30 minutes. The maximum vacuum drawn over water was 28.5 inches of mercury. The loss in moisture content of the sections during evacuation in air was not large. Some test specimens at 24 percent moisture content lost only 3 percent of moisture during a 20-minute evacuation.

After the evacuation period the sections were allowed to remain under water for 15 minutes or more before they were placed in the apparatus. Care was taken to avoid trapping any air underneath the section during this placement. The sections were so oriented that the retainer bars were at right angles to the longitudinal axis (across the tracheids) in order to maintain the maximum strength properties of the section. The disks were then drawn together, the levels in the burettes were adjusted, and pressure was applied.

Distilled water was used throughout the experiments.

EXPERIMENTAL RESULTS

RADIAL PERMEABILITY AT A PRESSURE OF 10 CM OF MERCURY

Less than one-half of the woods used in this study were appreciably permeable to water radially at a pressure of 10 cm of mercury (table 1). Although a measurable quantity of liquid flowed through these woods none of them showed a high degree of permeability when judged by the ease of liquid flow in the longitudinal direction. This was to be expected because the movement of liquid would be at right angles to the cell axis.

One of the most important facts shown by table 1 is that all the woods except one which were permeable at low pressure were the resinous woods or those which normally possess resin canals longitudinally and radially.[3] With nonresinous conifers an appreciable rate of flow was not obtained during a period of 15 minutes. There may be one exception to this statement, however, and that is balsam fir sapwood. In some determinations the data of which are not reported in table 1, a small rate of flow was obtained at low pressure (10 cm of mercury). These sections were from a different bolt of wood and the treatment of the wood before the sections were cut was not definitely known. Hence the inclusion of the results did not appear advisable. Nevertheless, it may be that some samples of balsam fir sapwood are permeable at low pressure.

Another fact of major importance shown in table 1 is that in almost all cases only the sapwood of the species represented was permeable at low pressure. The springwood of jack pine heartwood was an exception, showing almost the same rate of flow as the sapwood. That this may have been due to chance to some extent is indicated by the greater variation between the individual sections as shown by maximum and minimum final rates of flow in the last two columns of table 1. Some sections of tamarack and Douglas fir heartwoods showed a small rate of flow at low pressure, but many did not; consequently, they were all run only at high pressure.

The more permeable woods were the sapwoods of the southern pines, white spruce, tamarack, and Douglas fir. The variation in the rate-of-flow values for the individual sections of a given wood was of reasonably limited range in most woods. The last two columns

[3] Throughout this paper the term "resinous wood" refers to a wood which possesses a system of resin canals.

give the minimum and the maximum rates of flow at the end of 3 hours of continuous flow.

In general, the rate of flow became less as the time of continuous flow increased. The initial rate of flow was greater than that at any succeeding period except with tamarack sapwood, and the springwood of the sapwood of loblolly pine and white spruce. On an average, the final rate of flow for most woods was about one-third to one-fourth of the initial rate.

It is noticed from table 1 that in several woods the springwood was more permeable radially than the summerwood. The two rates of flow in the southern pines were in the ratio of 4 to 1. This difference is not very great, and the limited sampling reduces the significance of such differences. White spruce, Norway pine, and jack pine were about as permeable in the springwood as in the summerwood at the lower pressure.

TABLE 1.—*Radial rate of flow of water through thin seasoned sections of wood at different pressures*

PRESSURE, 10 CM MERCURY

Kind of wood	Sections	Average thickness	Estimated amount of summerwood	Rate of flow per minute after—					Minimum final flow	Maximum final flow
				0 hours	0.5 hour	1 hour	2 hours	3 hours		
	Number	*Millimeters*	*Percent*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Jack pine sapwood	4	1.28	0	0.00364	0.00284	0.00228	0.00187	0.00169	0.00118	0.00192
	6	1.20	50	.00920	.00378	.00339	.00223	.00197	.00128	.00345
Jack pine heartwood	5	1.26	0	.02302	.00512	.00360	.00196	.00148	.0000	.0048
Loblolly pine sapwood	4	1.27	0	.0231	.0660	.0555	.0438	.0389	.0205	.0563
	4	1.28	100	.0461	.0217	.0159	.0116	.0095	.0024	.0273
Longleaf pine sapwood	6	1.31	0	.0934	.0590	.0496	.0425	.0388	.0087	.0953
	6	1.29	100	.0260	.0179	.0146	.0146	.0122	.0024	.0273
Norway pine sapwood	4	1.29	0	.00343	.00221	.00169	.00124	.00125	.00070	.00277
	4	1.27	60	.00253	.00154	.00110	.00090	.00082	.00050	.00105
Shortleaf pine sapwood	4	1.29	0	.1315	.0496	.0371	.0303	.0264	.0028	.0482
	4	1.27	100	.03430	.0147	.0109	.0076	.0059	.0031	.0090
Slash pine sapwood	4	1.27	0	.529	.374	.301	.248	.235	.118	.556
	4	1.30	100	.163	.1022	.0791	.060	.0526	.0467	.0545
Tamarack sapwood	7	1.25	30	.381	.648	.548	.418	.354	.182	.588
Black spruce sapwood	5	1.29	0	.0013						
	4	1.26	20	.0010						
White spruce sapwood	8	1.24	0	.1405	.0662	.1514	.0442	.0326	.0092	.0790
	4	1.21	30	.0694	.0430	.0363	.0330	.0304	.0028	.0889
Douglas fir (coast type) sapwood	8	1.29	30	.0650	.0396	.0510	.0296	.0246	.0004	.0861
White oak sapwood	4	1.24	90	.00718	.00569	.00475	.00381	.00319	.00152	.00540

PRESSURE, 100 POUNDS PER SQUARE INCH

Jackpine sapwood	4	1.28	0	0.812	0.728	0.626	0.511	0.455	0.248	0.556
	5	1.21	50	.949	.574	.607	.488	.415	.179	.724
Jackpine heartwood	5	1.26	0	.959	.407	.301	.215	.181	.0151	.0541
	5	1.25	50	.2704	.0746	.0475	.0271	.0211	.0013	.0381
Loblolly pine sapwood	4	1.29	0	10.86	8.74					
	4	1.31	100	8.66	6.53					
Loblolly pine heartwood	3	1.25	0	1.326	.509	.416	.305	.259	.069	.385
	6	1.26	50	.0417	.0404	.0918	.0860	.0694	.0003	.370
Longleaf pine sapwood	4	1.28	0	7.08	6.19	5.98	5.69	5.49	1.05	13.3
	3	1.29	100	4.77	4.22	3.55	3.01	2.90	1.38	4.79
Longleaf pine heartwood	15	1.27	40	.0246	.0102	.008	.0058	.0046	.0000	.0278
Norway pine sapwood	4	1.29	0	.678	.597	.514	.413	.363	.204	.594
	4	1.29	60	.600	.571	.493	.403	.359	.115	.526
Norway pine heartwood	4	1.31	0	.1528	.0637	.0483	.0249	.0198	.0020	.0392
	5	1.29	40	.0176	.0055	.0046	.0030	.0021	.0000	.0057
Shortleaf pine sapwood	4	1.29	0	15.0						
	4	1.27	100	23.7						
Slash pine sapwood	4	1.27	0	22.4						
	4	1.30	100	18.4						
Tamarack heartwood	15	1.30	0	.656	1.56	1.63	1.70	1.67	.0000	7.52
	18	1.28	70	.414	.518	.544	.497	.442	.0000	2.73

TABLE 1.—*Radial rate of flow of water through thin seasoned sections of wood at different pressures*—Continued

PRESSURE, 100 POUNDS PER SQUARE INCH—Continued

Kind of wood	Sections	Average thickness	Estimated amount of summerwood	Rate of flow per minute after—					Minimum final flow	Maximum final flow
				0 hours	0.5 hour	1 hour	2 hours	3 hours		
	Number	*Millimeters*	*Percent*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Black spruce sapwood	8	1.27	0	.475	1.18	1.15	1.15	1.10	.321	2.50
	8	1.27	20	.570	1.07	1.204	1.38	1.56	.070	4.62
Black spruce heartwood	9	1.30	0	.0037	.0228	.0334	.0421	.0443	.0000	.377
	9	1.27	25	.0033	.0404	.0367	.0095	.0086	.0000	.0267
White spruce sapwood	4	1.21	30	6.43	1.34	1.16	1.47	.689	.235	1.17
White spruce heartwood	9	1.27	0	.190	.202	.191	.174	.163	.0026	.800
	10	1.24	30	.0139	.008	.0053	.0061	.0059	.0000	.0267
Eastern hemlock sapwood	8	1.23	30	.0401	.0199	.0163	.0119	.0101	.0033	.0154
Eastern hemlock heartwood	6	1.23	30	.0021	.0015	.0012	.0009	.0008	.0002	.0015
Western hemlock sapwood	4	1.23	25	.0197	.0090	.0073	.0061	.0056	.0025	.0103
Western hemlock heartwood	5	1.22	20	.0120	.0075	.0059	.0045	.0040	.0004	.0108
Douglas fir (coast type) sapwood	8	1.29	30	5.22	4.67	4.57	4.55	4.50	.072	9.52
Douglas fir (coast type) heartwood	10	1.26	0	.233	.361	.284	.231	.217	.000	.654
	9	1.34	85	.665	.593	.493	.371	.348	.004	.870
Balsam fir sapwood	6	1.24	0	.0366	.0214	.0197	.0185	.0173	.0027	.0289
	6	1.27	35	.0307	.0214	.0195	.0183	.0177	.0089	.0370
Balsam fir heartwood	7	1.27	0	.0031	.0017	.0015	.0013	.0011	.0000	.0025
	6	1.25	30	.0004	.0002	.0002	.0002	.0001	.0000	.0008
Northern white cedar sapwood	7	1.23	20	.0327	.0293	.0278	.0273	.0269	.0013	.0500
Northern white cedar heartwood	11	1.22	25	.0009	.0010	.0010	.0010	.0010	.0000	.0028
Paper birch sapwood	6	1.22	90	.0180	.0105	.009	.0065	.0061	.0040	.0083
Paper birch heartwood	5	1.22	90	0	0	0	0	0	0	0
White oak sapwood	4	1.24	90	.2243	.1305	.0988	.0528	.0407	.0220	.0674
White oak heartwood	4	1.30	100	0	0	0	0	0	0	0

RADIAL PERMEABILITY AT A PRESSURE OF 100 POUNDS PER SQUARE INCH

The data obtained on the radial rate of flow of water through sections of seasoned wood at a relatively high pressure, 100 pounds per square inch are also presented in table 1. These data show great variation in rate of flow among the woods tested. All of the woods except paper birch heartwood and white oak heartwood were permeable at high pressure. Among the most permeable woods were the southern pine sapwoods and Douglas fir sapwood, which had initial rates of flow of from 5 to 30 cc per minute. Those woods that were permeable at low pressure were also the most permeable at high pressure. They include the sapwoods and a few heartwoods of the species with resin canals. A cursory inspection of table 1 will show that for most woods the rate of flow was not of a high order of magnitude, usually only a few tenths of a cubic centimeter or less.

Whenever it was possible, springwood permeability was compared with summerwood permeability. In a number of woods, however, the annual rings were not of sufficient width to permit the cutting of springwood sections of the desired thickness. The sections cut from these woods contained both springwood and summerwood, and in about the same proportions as the original block. The estimated amount of summerwood in the sections containing summerwood is given in table 1. Zero percent designates springwood. Percentages are admittedly not accurate but they represent an estimated average for each series of sections and give some idea of the amounts involved.

Of eight sapwoods tested at high pressure the majority showed about the same permeability in the springwood as in the summerwood. In no case was one part of the annual ring as much as twice as permeable as the other. By species, these sapwoods were: Jack pine, loblolly pine, longleaf pine, Norway pine, shortleaf pine, slash pine, black spruce, and balsam fir. Although the southern pine sapwoods were somewhat more permeable in the springwood at a pressure of 10 cm of mercury this apparently was doubtful at 100 pounds per square inch.

In contrast to this, the springwood of the heartwoods was usually more permeable than the summerwood. The one exception was Douglas fir, which was about as permeable in the springwood as in the summerwood. The springwood of loblolly pine, tamarack, and black spruce heartwoods was 4 or 5 times as permeable as the summerwood; that of jack pine, Norway pine, and balsam fir heartwoods about 10 times as permeable; and that of white spruce nearly 30 times as permeable.

It is desirable when data of this sort are available to make a comparison of the various woods on the basis of their permeability as indicated by rate-of-flow measurements. In so doing two separate arrangements must be made—one including the springwood sections and one including the sections containing summerwood—because the average permeability of some woods was not determined and it may not be a simple average of the springwood and summerwood values or the summerwood values alone. The order of arrangement can be more properly given by dividing the list of woods into groups which are determined in their limits by a shift of one decimal place in relation to the first significant number of the rate-of-flow values.

The order of the woods on the basis of their observed springwood permeability (from table 1) is as follows:

Southern pine sapwoods Tamarack heartwood Black spruce sapwood	1 cc per minute or more.
Jack pine sapwood Norway pine sapwood Loblolly pine heartwood Douglas fir heartwood Jack pine heartwood White spruce heartwood	Range, 0.5 to 0.1 cc per minute.
Black spruce heartwood Norway pine heartwood Balsam fir sapwood	Range, 0.04 to 0.01 cc per minute.
Balsam fir heartwood	0.001 cc per minute.

A similar list based on data from the sections containing summerwood of the amounts indicated in table 1 includes all the species studied. The list in the order of decreasing final rate of flow through the woods is as follows:

Southern pine sapwoods (on basis of initial rate of flow) Douglas fir sapwood Black spruce sapwood	1.5 cc per minute or more.
White spruce sapwood Tamarack heartwood Jack pine sapwood Norway pine sapwood Douglas fir heartwood	Range, 0.7 to 0.3 cc per minute.

Loblolly pine heartwood White oak sapwood Northern white cedar sapwood Jack pine heartwood Balsam fir sapwood Eastern hemlock sapwood	Range, 0.07 to 0.01 cc per minute.
Black spruce heartwood Paper birch sapwood White spruce heartwood Western hemlock sapwood Longleaf pine heartwood Norway pine heartwood	Range, 0.009 to 0.002 cc per minute.
Northern white cedar heartwood Eastern hemlock heartwood Balsam fir heartwood White oak heartwood Paper birch heartwood	Range, 0.0009 to 0.00 cc per minute.

The above classifications are not to be considered as absolute or fixed orders of arrangement but only as an approximate classification in which a wood of one group may belong to another group when different samples or different experimental conditions are employed.

In almost every species the radial permeability of the sapwood was greater than that of the heartwood. In no case was a greater heartwood permeability found, but in two species the heartwood and sapwood permeabilities were not very different; western hemlock sapwood was less than one and one-half times as permeable as heartwood, and the springwood of jack pine sapwood was more than twice as permeable as the springwood of jack pine heartwood. In making such comparisons from the data of table 1, it is obvious that where springwood and summerwood permeabilities were measured separately comparisons of heartwood and sapwood also should be made separately and on the same part of the annual ring.

The approximate ratio of sapwood to heartwood permeability for other species based on springwood, summerwood, or both together is as follows: Jack pine summerwood, 20; Norway pine springwood, 18, summerwood, 170; black spruce springwood, 25, and summerwood, 180; white spruce summerwood, 120; eastern hemlock, 13; western hemlock, 1; Douglas fir summerwood, 13; balsam fir springwood, 16, and summerwood, 130; northern white cedar, 27. Paper birch and white oak heartwoods were practically impermeable so numerical comparison is impossible.

Where comparisons are made on the basis of summerwood sections, it is not meant that the sections were entirely of summerwood; they contained only the amount of summerwood indicated in the fourth column of table 1, which was not always the same for heartwood and sapwood. Considering the small sampling and from only one log, the figures are approximations.

A decrease in rate of flow as the time of continuous flow increased was again the general occurrence, but in this series of tests a number of woods gave increased rates of flow with time as measured by a comparison of initial and final rate of flow. The following woods gave a final rate of flow of water which was from one to three times the initial rate of flow for the same woods: Summerwoods of loblolly pine, tamarack, and northern white cedar heartwoods, and of black spruce heartwood and sapwood and springwoods of tamarack heartwood, black spruce sapwood, and Douglas fir heartwood. In several of these woods, namely, the summerwoods of northern white cedar, tamarack,

and Douglas fir heartwoods, the initial rate was but little different from the final rate of flow. The period of time over which flow continued to increase varied with the kind of wood. The summerwood of black spruce sapwood increased in permeability to water to the very end of the run. In tamarack heartwood the maximum rate of flow was reached after 1 to 2 hours of flow. In other woods, the maximum was reached after ½ to 1 hour.

The sections of a wood did not always behave identically either in increasing or decreasing the rate of flow. Occasionally both processes occurred simultaneously in different sections. The reported values therefore are merely the net result. In some instances the rate of flow fluctuated considerably in the same section during the run. This sometimes occurred in black spruce heartwood and sapwood and in some sections of tamarack and of white spruce sapwood. In several sections of black spruce heartwood the rate of flow increased only slightly during the first 15 minutes or half hour and then suddenly increased very greatly, after which it continued to increase slowly in some of the sections and began to decrease in others. Almost every type of flow situation was found in a few of these resinous woods.

The nonresinous woods did not show these abnormalities in rate of flow. Northern white cedar showed a delayed flow in some sections, but the rate of flow was very small and no striking fluctuations or changes occurred as in the above cases.

Attention should be called to the degree of variation in radial permeability between individual sections of a given wood. Some idea of the variation obtained may be gained by comparing the last three columns of the latter part of table 1, which show the average final rate of flow (after 3 hours), the minimum final rate of flow, and the maximum final rate of flow in the order given. The more resistant woods, in general, show considerable variation. Tamarack heartwood seemed to be the most inconsistent of the group. Some sections cut from the same small piece were very resistant and occasionally showed no rate of flow, whereas a few were very permeable. This was true of the 16 and 18 sections of springwood and summerwood of tamarack heartwood which were tested. In several other woods some sections were apparently impermeable by the methods used. Variations in which the maximum value was 10 times the minimum value were not uncommon, and in a few instances the difference was much greater.

TANGENTIAL PERMEABILITY

The data obtained from experiments on the tangential permeability of the various woods are given in table 2. It is readily seen that the magnitude of the rate of flow is much less on an average than the radial rate of flow (a comparison is presented in table 3). A number of the heartwoods appeared to be impermeable when judged by the technique of this investigation.

The more permeable woods, which generally were the sapwoods, were tested at a pressure of 10 cm of mercury before shifting to the high pressure. A 15-minute period was allowed for the flow of water to manifest itself; if no easily measurable flow (0.005 cc) occurred within that time, the wood was considered to be impermeable by this technique and at this pressure. Only two woods were found through which the flow of water was appreciable—loblolly pine and slash pine sapwoods. Of these two the slash pine was the more permeable and

the average final rate of flow through the sections was 0.003 cc per minute. The rate of flow through loblolly pine sapwood at the end of 1 hour was only 0.0002 cc per minute.

TABLE 2.—*Tangential rate of flow of water through thin sections of seasoned wood at a pressure of 100 pounds per square inch*

Kind of wood	Sections	Average thickness	Estimated amount of summerwood	Rate of flow per minute after—					Minimum final flow	Maximum final flow
				0 hour	0.5 hour	1 hour	2 hours	3 hours		
	Number	*Millimeters*	*Percent*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Jack pine sapwood	4	1.32	25	0.00314	0.00303	0.00326	0.00268	0.00245	0.00154	0.00364
Jack pine heartwood	6	1.26	25	0	0	0	0	0	0	0
Loblolly pine sapwood	3	1.33	60	.0541	.0461	.0408	.0379	.0335	.0286	.0405
Loblolly heartwood	4	1.32	30	0	0	0	0	0	0	0
Longleaf pine sapwood	6	1.34	40	.0375	.0294	.0266	.0221	.0176	.0099	.0357
Longleaf pine heartwood	4	1.32	30	0	0	0	0	0	0	0
Norway pine sapwood	4	1.29	25	.0165	.0124	.0108	.0096	.0086	.0068	.0108
Norway pine heartwood	5	1.28	20	0	0	0	0	0	0	0
Shortleaf pine sapwood	5	1.35	40	.1442	.117	.0969	.0814	.0728	.0287	.1764
Slash pine sapwood	3	1.36	50	.408	.363	.297	.253	.223	.076	.313
Tamarack sapwood	4	1.32	25	.00297	.00273	.00251	.00231	.00215	.00167	.01020
Tamarack heartwood	4	1.32	30	.00199	.00243	.00175	.00159	.00134	.0000	.00323
Black spruce sapwood	4	1.34	15	.00289	.00307	.00289	.00273	.00263	.00204	.00333
Black spruce heartwood	4	1.31	15	.00026	.00012	.00012	.00011	.00010	.00000	.00175
White spruce sapwood	4	1.34	15	.00072	.00158	.00161	.00152	.00150	.00133	.00179
White spruce heartwood	6	1.34	15	.00006	.00008	.00007	.00009	.00011	.0000	.00042
Eastern hemlock sapwood	6	1.31	20	.02753	.01236	.00938	.00638	.00560	.00213	.00923
Eastern hemlock heartwood	4	1.27	20	.00779	.00523	.00404	.00343	.00318	.00090	.00555
Western hemlock sapwood	5	1.27	15	.02531	.00968	.00629	.00440	.00399	.00245	.00545
Western hemlock hardwood	5	1.29	15	.03703	.01980	.01556	.01033	.00838	.00250	.01429
Douglas fir sapwood	7	1.28	30	.00005	.00026	.00075	.00071	.00075	.0000	.00150
Douglas fir heartwood	6	1.30	25	.00040	.00036	.00033	.00036	.00032	.0000	.00106
Balsam fir sapwood	6	1.31	15	.0185	.0139	.0120	.0095	.0081	.0038	.0241
Balsam fir heartwood	8	1.31	15	.00022	.00018	.00017	.00014	.00012	.0000	.00060
Northern white cedar sapwood	4	1.29	10	.0577	.0494	.0478	.0440	.0431	.0397	.0508
Northern white cedar heartwood	4	1.26	10	.0177	.0168	.0162	.0126	.0113	.0024	.0230
Paper birch sapwood	4	1.37	90	.00172	.00253	.00244	.00210	.00201	.00136	.00263
Paper birch heartwood	4	1.31	90	0	0	0	0	0	0	0
White oak sapwood	4	1.37	70	.00876	.00711	.00624	.00583	.00550	.00400	.00796
White oak heartwood	4	1.35	70	0	0	0	0	0	0	0

The phenomenon of decreasing rate of flow with increasing time of continuous flow and a gradual approach to equilibrium was likewise observed in the tangential flow of liquids through wood. In general, the decrease in flow with time was less than was found for radial permeability. For most woods the final rate of flow was from one-half to three-fourths of the initial rate. In four cases the final rate was somewhat greater than the initial rate; these were white spruce sapwood and heartwood, Douglas fir sapwood, and paper birch sapwood. Black spruce sapwood had about the same initial and final rate-of-flow values. The woods in which the final rate was from four-fifths to three-fifths of the initial rate includes jack pine sapwood, tamarack heartwood and sapwood, Douglas fir heartwood, northern white cedar sapwood and heartwood, white oak sapwood, and loblolly pine sapwood. A final rate of flow of about one-half that of the initial flow was found with longleaf pine, shortleaf pine, slash pine, and Norway pine sapwoods, balsam fir heartwood and sapwood. In the remaining woods the final permeability was from about one-third to one-tenth of the initial value. These woods are: Black spruce heartwood,

western hemlock heartwood and sapwood, and eastern hemlock sapwood and heartwood.

These fractional values are not to be regarded as strictly accurate. One reason for this is that much depends upon obtaining the volume of flow during the first few minutes of flow in order that the value may truly represent the initial rate of flow. With woods of very low permeability it was difficult to measure the volume of water coming through the section in only 2 or 3 minutes; hence the period of a measurement was extended to the time necessary to give a fairly accurate volumetric reading. The extra few minutes would tend to change the value somewhat because the reported value would be a near-average of the flow during the total time of the reading during which the rate of flow is usually simultaneously decreasing. This correction also applies for similar cases in radial flow measurements.

A sound reason for caution in any interpretation of a comparison of final and initial rates of flow is that several of the woods increased in permeability during the first 30 minutes or more before the decrease in rate of flow became evident. This group was represented by tamarack heartwood, and the sapwoods of black spruce, white spruce, and paper birch. Douglas fir sapwood and white spruce sapwood were more peculiar in that some sections immediately increased in apparent permeability for a time, then decreased. Other sections did not begin to conduct liquid until 1 or 2 hours had elapsed. They then continued to increase in permeability and thereby offset the declining rate of a few other sections. The result was that the reported data show minor fluctuations or almost constancy over the several intervals of time.

It is not difficult to establish comparisons between heartwood and sapwood permeabilities in the tangential direction. Table 2 shows that in the heartwood of six species either no flow occurred or only an indeterminate amount of water passed through the sections. In these woods, therefore, the moderately permeable sapwood was infinitely more permeable than the heartwood. Such was the case for jack pine, loblolly pine, longleaf pine, Norway pine, paper birch, and white oak. In tamarack, eastern hemlock, and Douglas fir the sapwood was about twice as permeable as the heartwood, in northern white cedar 4 times, in white spruce 15 times, in black spruce 25 times, and in balsam fir 70 times. The one exception was western hemlock, in which the heartwood was twice as permeable as the sapwood. The significance of such a small difference is seriously questioned when the individual variation, shown by the last two columns in table 2, is taken into consideration. This qualification also applies to the three woods in which the sapwood was twice as permeable as the heartwood.

The data in table 2 permit a direct comparison of the tangential permeabilities of the woods. The thickness of the sections is not the same for all woods but is not sufficiently different to affect the results in a general way. The estimated amount of summerwood in the sections is, of course, in the same proportion as in the original piece of wood because of the radial direction of sectioning it.

When the woods are arranged in the order of decreasing permeability and divided into groups the limits of which are arbitrarily fixed

by a shift of one decimal place to the first significant number in the final rate-of-flow values, the list is as follows:

Slash pine sapwood	0.2 cc per minute.
Shortleaf pine sapwood	Range, 0.09 to 0.01 cc per minute.
Northern white cedar sapwood	
Loblolly pine sapwood	
Longleaf pine sapwood	
Northern white cedar heartwood	
Norway pine sapwood	Range, 0.009 to 0.001 cc per minute.
Western hemlock heartwood	
Balsam fir sapwood	
Eastern hemlock sapwood	
White oak sapwood	
Western hemlock sapwood	
Eastern hemlock heartwood	
Black spruce sapwood	
Jack pine sapwood	
Tamarack sapwood	
Paper birch sapwood	
Tamarack heartwood	
Douglas fir sapwood	Range, 0.0009 to 0.0001 cc per minute.
Douglas fir heartwood	
Balsam fir heartwood	
White spruce heartwood	
Black spruce heartwood	
Jack pine heartwood	No apparent flow of water.
Loblolly pine heartwood	
Longleaf pine heartwood	
Norway pine heartwood	
Paper birch heartwood	
White oak heartwood	

The variation between individual sections of a given set of sections was considerably less, on an average, for tangential flow than for radial flow. The last two columns of table 2 show the maximum and minimum final rates of flow for the sections of a given wood. In six woods some sections were apparently impermeable and others of the same wood were somewhat permeable. However, as can be seen from table 2, these sections were not of a high degree of permeability.

In the sapwoods of nine species the maximum values were from about one to three times the minimum rates of flow. This represents a fair degree of uniformity. In three woods the maximum final value was from about 3 to 4 times the minimum; in three woods it was 6 times the minimum, and in one wood it was 10 times the minimum value. Other woods had zero for a minimum. This greater uniformity as compared to the radial flow values was found in both the resinous and nonresinous woods. The woods which exhibited the least variation were: The sapwoods of northern white cedar, paper birch, white oak, jack pine, loblolly pine, Norway pine, black spruce, white spruce, and western hemlock.

In order to determine whether higher pressure would have a deleterious effect on the sections, the pressure was increased to 150 pounds per square inch on several of the woods after the regular 3-hour run at 100 pounds per square inch. In longleaf pine heartwood one section showed a flow of 0.006 cc in 30 minutes and the other three showed no flow. In loblolly pine heartwood only one section showed a very small flow of water and the same was true with four sections of black spruce. In the latter the same section was the only one that permitted flow at both pressures. Two sections each of balsam fir and white spruce heartwoods were tested in the same way. Only

one section of each kind had given a measurable flow at the usual pressure, but at 150 pounds per square inch they increased in rate of flow from one to two times and the other two then revealed a small but definite permeability to water in order of one or two ten-thousandths of a cubic centimeter per minute. This shows that a pressure of 100 pounds per square inch probably did not have a harmful effect on the sections of wood of the thickness used by causing microscopic rupture.

COMPARISON OF RADIAL AND TANGENTIAL PERMEABILITY

The data of tables 1 and 2 afford a basis for comparing in a qualitative way the radial and tangential permeability of a given wood. To enable such a comparison to be made easily, the essential data have been combined in table 3.

TABLE 3.—*Comparison of radial and tangential rates of flow through thin sections of seasoned wood at a pressure of 100 pounds per square inch*

Kind of wood	Radial		Tangential	
	Estimated amount of summerwood	Flow per minute after 3 hours	Estimated amount of summerwood	Flow per minute after 3 hours
	Percent	*Cc*	*Percent*	*Cc*
Jack pine sapwood	0	0.455	25	0.00245
	50	.415		
Jack pine heartwood	0	.181	25	0
	50	.0211		
Loblolly pine sapwood	0	[1] 10.9	60	.0335
	100	[1] 8.66		
Loblolly heartwood	0	.259	30	0
	50	.0694		
Longleaf pine sapwood	0	5.49	40	.0176
	100	2.90		
Longleaf pine heartwood	40	.00458	30	0
Norway pine sapwood	0	.363	25	.00858
	60	.359		
Norway pine heartwood	0	.0198	20	0
	40	.0021		
Shortleaf pine sapwood	0	[1] 15.0	40	.0728
	100	[1] 23.7		
Slash pine sapwood	0	[1] 22.4	50	.223
	100	[1] 18.4		
Tamarack sapwood			25	.00215
Tamarack heartwood	0	1.67		
	70	.442	30	.00134
Black spruce sapwood	0	1.096	15	.00263
	20	1.567		
Black spruce heartwood	0	.0443	15	.00010
	25	.0086		
White spruce sapwood	30	.689	15	.00150
White spruce heartwood	0	.1630	15	.00011
	30	.0059		
Eastern hemlock sapwood	30	.0101	20	.00560
Eastern hemlock heartwood	30	.00077	20	.00318
Western hemlock sapwood	25	.00561	15	.00399
Western hemlock heartwood	20	.0040	15	.00838
Douglas fir (coast type) sapwood	30	4.50	30	.00075
Douglas fir (coast type) heartwood	0	.217	25	.00032
	85	.348		
Balsam fir sapwood	0	.0173	15	.00812
	35	.0177		
Balsam fir heartwood	0	.00109	15	.00012
	30	.00013		
Northern white cedar sapwood	20	.0269	10	.0431
Northern white cedar heartwood	25	.00098	10	.0113
Paper birch sapwood	90	.0061	90	.00201
Paper birch heartwood	90	0	90	0
White oak sapwood	90	.0407	70	.0055
White oak heartwood	100	0	70	0

[1] Initial rate of flow.

In the columns under radial flow, both the final rate-of-flow values of sections containing only springwood and of sections containing summerwood (or springwood and summerwood) are given for the woods in which they were measured separately. The question may arise as to whether the tangential rate of flow should be compared with the springwood value of radial flow for the species or with the summerwood value. The correct answer can only be that the presence of three types of permeability must be recognized: (1) One involving the average of the permeabilities of springwood and summerwood acting independently of each other (in tangential flow); (2) one involving only the springwood radial permeability; and (3) one involving either the summerwood radial permeability alone or the resultant radial permeability of springwood and summerwood acting together in series, depending upon whether or not the section is entirely of summerwood.

In these cases, therefore, tangential rate of flow must be compared separately with the springwood and the summerwood radial rates of flow. With many woods the results will be about the same, for it has already been pointed out that, in the sapwoods, radial rate of flow through the springwood is about the same as that through the summerwood, but in most of the heartwoods this is not true.

Then there is also the fact that where the tangential permeability is compared to the radial permeability of sections containing summerwood, the percentage of summerwood is seldom the same in the two types of sections. One cannot say whether a smaller percentage of summerwood in the radial sections (for tangential flow) than in the tangential sections favors greater tangential permeability, for this depends on the relative permeability of springwood and summerwood in the tangential direction, and on this subject no accurate information is available. It is evident from the above discussion that many comparisons of radial and tangential permeability based on the data of table 3 will at best be only approximations because of the variability of wood, the lack of average values for some woods, the often different amounts of summerwood in the radial and tangential sections, and the possible difference between the average thicknesses of any two sets of sections under consideration. The last item is probably of minor importance as compared with variations in sampling.

Probably the most obvious conclusion from table 3 is the lower average value for tangential flow through the woods as compared to the average radial value determined merely by inspection. Further analysis reveals that this was because of much smaller tangential permeability values for the resinous woods. In fact, several of the heartwoods of the resinous woods were apparently impermeable, thereby exhibiting a sharp contrast to their radial permeability. The resinous sapwoods were also much less permeable tangentially than radially. The nonresinous woods, on the other hand, were about as permeable radially as tangentially on an average.

Aside from those heartwoods which were permeable radially but not tangentially, most of the other resinous woods also showed very decided differences. Jack pine sapwood was nearly 200 times as permeable radially as tangentially, longleaf pine sapwood 160 to 310 times as permeable (based on summerwood and springwood respectively), Norway pine sapwood 40 times, tamarack heartwood 330 to 1,200 times, black spruce sapwood 400 to 600 times, black spruce

heartwood 90 to 440 times, white spruce sapwood 450 times, white spruce heartwood 50 to 1,500 times, Douglas fir sapwood 6,000 times and the heartwood about 1,000 times as permeable.

The differences between the rates of flow in the two directions for the nonresinous woods were not nearly so extreme. Western hemlock heartwood and sapwood, balsam fir sapwood, and the sapwoods of eastern hemlock, paper birch, and northern white cedar were as permeable radially as tangentially or favored either direction by from one to three times. The smaller differences are of doubtful significance. White oak sapwood was about seven times as permeable radially and balsam fir heartwood springwood nine times as permeable radially as the tangentially. The tangential rate of flow through balsam fir heartwood was the same as the radial rate of flow through the summerwood of the heartwood. However, eastern hemlock and northern white cedar heartwoods were respectively 4 and 11 times as permeable tangentially as radially.

Even though these ratios are to be considered as approximations, they very clearly show the marked difference between radial and tangential permeabilities of the woods possessing resin ducts and the relatively small or negligible differences between the two directional permeabilities of the woods without resin ducts.

DISCUSSION

The results of these investigations represent an attempt to establish a number of fundamental relationships of wood permeability which have hitherto been in controversy or have received little or no experimental analysis. In addition, the results have served to verify work which has already been fairly well accepted and at the same time to call attention to cases in which the general rule may not hold true.

There is now little room for doubt that a decrease in permeability with increased time of continuous flow is an almost universal phenomenon in the flow of liquids through wood. The results of this work and that of others (*5*, *6*, *12*, *23*) are evidence for this statement. The number of exceptions to this rule are few. The fact that in some cases an increase in rate of flow is obtained before the decrease begins does not invalidate this conclusion, for it means that other factors are of greater immediate effect and hence outweigh the tendency toward a decrease in rate of flow. The cause of such an increase during the first part of the run is not clearly understood. It was reported in a previous paper (*6*) that surface tension effects were of importance in certain seasoned woods as determined by the effect of evacuation on several heartwoods. All degrees of variation were obtained, depending upon the kind of wood and its resistance to the flow of water. Failure to evacuate the sections sometimes caused the rate of flow to remain the same for a period of time and sometimes resulted in a temporary increase in rate of flow over the initial rate, which was generally lower for the unevacuated sections in such a situation. This was ascribed to the surface tension of liquid in the capillary openings. It seems reasonable to assume that surface tension might have a similar effect on radial and tangential flow. It has been pointed out by Hawley (*9*) that although surface tension forces or capillary pressures of high magnitude may be developed in the small openings of the pit membrane they do not assist materially in the flow of liquid into wood

and may actually retard the rise of liquid in the coarse capillaries of the wood.

Since woods are very resistant radially, and especially tangentially, the effective capillaries must be few or small, or both. In the tangential direction, for example, the liquid must traverse 20 to 40 cells in passing through a section 1 mm thick. If meniscuses must be broken in each encounter with a cell, considerable time may be involved in so doing, for it was noticed with transverse sections that much of the air in a section of a moderately resistant wood was not forced out immediately. In tangential and radial sections evacuation may not be complete in the more resistant woods. If this air were trapped in the cell it would undoubtedly cause a meniscus to be formed at the openings in contact with water and thus block its own outward passage and the flow of water as well. In some situations the water may, because of its high pressure, dissolve considerable of the air and thus free the capillaries.

If a resistant wood is not filled with water by the process of evacuation, and this seemed to be the case in many woods, then some time is necessary for filling the void volume of the section even though it is only about 0.015 cc for the exposed portion of the average section and assuming for the moment that no surface-tension effects occur. The effective void volume is probably greater than this because the liquid would also tend to move into portions of the section other than the area exposed, particularly longitudinally; consequently the given figure might be too small for a resistant wood in which much water has not entered even after evacuation. Where the rate of flow is only of the order of a few ten-thousandths to a few thousandths cubic-centimeters per minute, this is a real situation and seems to apply to tangential flow through very resistant woods.

In radial permeability there is the possibility that, if flow occurs through resin ducts, particles of resin may be torn away and washed out with other loose fragments of resin. This would account for some sudden increases in flow observed in a few sections of white and black spruce.

Without further discussion, therefore, it may safely be said that the increasing rate of flow during the early part of the run that is sometimes noticed in such woods as black spruce and white spruce heartwoods or the long-delayed tangential flow in Douglas fir sapwood, is due to a number of complicating factors which probably are more or less related and often simultaneously effective and which are exceedingly difficult to evaluate.

The results of a comparison of radial and tangential permeabilities of the various woods indicate that radial flow occurs either through the resin canals or through the wood rays.

Microscopic examination of wood shows that the radial walls of tracheids are profusely supplied with bordered pits, the springwood cells having a greater number than the summerwood cells. On the tangential walls, pits are essentially absent in the springwood cells, but are present in most conifers, though usually few, in the last rows of summerwood cells. This may be verified by the literature (*11*).

If flow occurs through bordered pits, a greater rate of flow should be obtained in the tangential direction because of the large number of bordered pits on the radial wall even if a goodly percentage of them were aspirated. The data show, however, that this did not occur.

In fact, the reverse was true, for the woods with resin canals. There is a possibility that the water may follow a roundabout course in moving radially through the section by taking advantage of the unsymmetrical arrangement of cells which usually exists to some extent in springwood. In this case the water would pass through the radial wall into the lumen of an adjacent cell and out through the other side of the same radial wall into a third cell which, with the first cell, has part of its radial wall in contact with the second cell, somewhat in the fashion that bricks are laid. This is a possible mechanism because most of the woods have a double row of pits either rarely or frequently, and in such a situation are likely to have pits on the same wall leading to two different cells.

While this mechanism may be a possibility in the springwood, it is very unlikely in the summerwood because of the more regular and linear order of the cells tangentially, the narrower width radially, and fewer pits. Although pits are present in the tangential walls of the last few rows of summerwood of conifers, except in the hard pines, they probably do not assist in radial flow because there is no apparent way by which the liquid could traverse the entire summerwood or pass beyond into springwood cells. Then, too, the results are about the same for woods with or without tangential pits. Further proof that these pits must be of minor importance, if any, lies in the fact that in both jack pine sapwood, a resinous wood, and balsam fir sapwood, a nonresinous wood, the springwood and summerwood of each wood were equally permeable. If the zigzag or roundabout path of flow were of importance, springwood permeability should have been much greater than that of summerwood. Furthermore, tangential permeability should have been greater than radial permeability because of the greater number of openings, and the more direct path of flow. Only relatively few cells are appropriately arranged for liquid movement through the bordered pits in a radial direction.

It has already been stated that the radial permeability of the nonresinous woods was not very different on an average from the tangential permeability. Thus it appears from the above discussion that the liquid must flow through the wood rays in a radial direction and through the bordered pits of the radial walls in tangential movement.

The two most probable paths of radial flow in the resinous woods are woodrays and resin canals. The fact that the radial flow through these woods was much greater than the tangential flow, which was usually not true of the nonresinous woods, suggests that the added structural feature in the radial direction was chiefly responsible, namely, the resin canals. It is true that some of the species, the pines in particular, have more ray tracheids than the nonresinous woods (*3*), but the other species of both groups tend to discredit the importance of these structural features. For example, black spruce, white spruce, and Douglas fir all have only one row of marginal ray tracheids, yet these woods show great differences between radial and tangential permeabilities. Eastern hemlock and western hemlock also have one row of marginal ray tracheids and show little difference between their radial and tangential permeabilities. The radial rates of flow for the sapwoods of the above species both with and without resin canals are not even approximately the same, and they should be so if the amount of ray tracheid tissue were the important factor, even though allow-

ance is made for a variation of 100 percent in the total wood-ray cross-sectional area.

It is interesting to note, however, that there was a certain uniformity or agreement in values for radial permeability of the nonresinous sapwoods and the sapwoods of white oak and paper birch. The values read as follows in cubic centimeters per minute: 0.01, 0.006, 0.017, 0.006, 0.027, and 0.04. The two lowest values belong to western hemlock and paper birch sapwoods. The tangential permeability of paper birch and white oak sapwood was one-third and one-seventh, respectively, of the radial values.

Returning to a consideration of radial flow in the resinous woods, it is believed that the explanation offered by other workers best fits the experimental data. Tiemann (*27*) suggested that in the sapwood the resin canals may be open or clogged; Weiss (*28*) and Teesdale (*24*) attributed the greater resistance of the heartwoods to creosote impregnation to the harder and more insoluble resin in the resin canals of the heartwood which would close up the canals more effectively. This would explain the large sapwood permeability as compared with that of heartwood. The presence of tylosoids in the resin canals of the heartwood of some species (Norway pine, jack pine, and the four southern pines) would also hinder liquid flow through the canals if one assumes that they are effective in the conduction of liquid.

The peculiar variations in rate of flow of some resinous woods, as noted in the experimental results, is most easily explained on the basis of the effectiveness of the resin canals. Sudden increases in rate of flow might be due to the forcing out of particles of resin adhering to or clogging the canal. On the other hand, some particles might be broken loose but encounter larger particles and cause a jam with cumulative effects unless it were weakened in some way and flushed out. From the variations in rate of flow obtained in some woods, notably white spruce, black spruce, tamarack, and Douglas fir, this is a possible explanation. For one section of white spruce sapwood summerwood the rate of flow after 15 minutes was only 13 percent of the initial flow. At 1.5 hours the flow was 80 percent greater than at 1 hour, and after 2.5 hours the flow was again the same as at 1.5 hours. Sections of Douglas fir heartwood cut from the same piece were often very different in rate of flow, some showed only a slight permeability initially and stopped altogether after 0.5 to 1 hour, and others showed rates of flow a few tenths of a cubic centimeter or more.

It is an interesting fact that these woods which showed abnormal rate-of-flow curves at high pressure did not do so at 10 cm of mercury (table 1). High pressure seemed to bring out more variation and discrepancies within the same section or between sections of the same kind of wood.

Although the phenomenon of an increase in rate of flow preceding the usual decrease with time was found in tangential flow, it was always of a smaller or negligible magnitude, more regular, taken section for section, and less frequent by species than in radial flow.

If difference in rate-of-flow curves for the different sections is attributed to surface-tension effects of a water-air system in the wood, then the greater and more sudden variations in flow in radial direction as compared to the tangential direction again indicate that the capillaries are larger radially when freed from hindrances to flow of water, and are more easily opened up than the tangential capillaries, which

also should mean a larger and more direct capillary. Again, this points to resin canals because the flow through resinous woods radially was usually much greater than tangentially and greater also than radial flow through nonresinous woods.

The discussion so far has not taken into account the possible permeability of the cell wall itself, exclusive of the pit membranes. Considering the nature of the cell wall, its submicroscopic and microscopic structure, and the fact that the cell-wall substance in the direction across the fiber becomes of considerable thickness even in a thin section, it does not seem likely that cell-wall permeability is important in these experiments. A number of woods showed no apparent flow of water by the methods used. This is evidence that the cell wall itself was practically ineffective in conducting water as measured by this technique. Stamm (*21*) from calculations of his data found that the pit-membrane pores were far more important as a cause of permeability to flow under pressure in the three structural directions than cell-wall permeability. The latter was negligible by comparison except in extremely resistant woods.

The idea that resin canals function in the flow of liquid in wood is not a new one. A comprehensive review on this topic has been given in the review of literature. From this scattered and somewhat contradictory information it is concluded that with different conditions, woods, and direction of flow, the resin ducts may or may not function. The results of creosote impregnation tests at high temperature may be different than when water, lower temperature, and thin sections are used; nor may the results obtained longitudinally be always applicable to radial penetration. At any rate there is evidence in the literature that resin canals may transport liquid radially, although there is no proof that they are the sole means of radial movement. In this paper the possible mechanism of radial flow has been developed from more complete data than have hitherto been available and without the support of other experimental work, although it is seen that several of the ideas are in agreement.

The disturbing feature to the suggestion that wood rays are effective in radial flow lies in the counter reports in the literature. Teesdale and MacLean (*24*, *25*) found little or no creosote in the wood rays or other wood parenchyma of both hardwoods and softwoods. Bailey observed no entrance of carbon particles into the wood rays of several conifers (*2*). Stamm (*18*), however, noted that the effective area of tangential sections of several woods was about the same as that of the ray cells, but this can only be considered as indirect evidence of wood-ray permeability. In a later paper MacLean (*14*) states that "little is known about their influence on penetration," but that in some hardwoods whose vessels are plugged by tyloses the simple pits (of wood rays and other parenchyma) may aid in penetration.

Some qualitative evidence that the wood-ray cells are permeable was obtained by examining the tangential sections of southern pine near the edges where they were sealed with paraffin. Under low-power magnification the colored paraffin was seen in the wood rays apparently in some rows of cells and not in others. A detailed examination was not possible, but the observation warrants further investigation into the problem of wood-ray penetrability.

Concerning the relative penetrability in three structural directions, penetration is considered to be greatest longitudinally, and radial or

tangential precedence is variable. The radial penetration is reported by MacLean (*14*) to be greater in some pines, but the tangential penetration in most of the other conifers is usually greater except in some resistant species where the difference is small. In the present series of experiments, tangential permeability greater than or equal to the radial permeability was unusual in resinous woods, but the contrary was fairly common in the nonresinous woods.

The results of investigations on creosote impregnation of wood by Weiss (*28*) and Teesdale (*24*) agree fairly well in a qualitative way with the results reported in this paper on the comparison of radial and tangential permeabilities. In resinous woods they found greater penetration of creosote radially than tangentially and in nonresinous woods the penetrations were about equal. Sutherland, Johnston, and Maass (*23*) found about equal permeability radially and tangentially in seasoned white spruce sapwood. In this work, however, the radial flow was over 400 times the tangential flow. The statement by Johnston and Maass (*12*) that jack pine sapwood is of the same order of permeability in the three structural directions is undoubtedly in error. Their conclusions were based on only one test in each direction.

Several exploratory experiments by Sutherland, Johnston, and Maass (*23*) indicated that in the two species tested the radial and tangential rates of flow were from 2 percent to less than 1 percent of the longitudinal rate of flow. Creosote penetration measurements (*24*, *28*) are of the order of 10 to 100 times greater penetration longitudinally than tangentially, with the radial comparison ratio usually somewhat less.

Comparisons of the rate of flow in the three structural directions were not determined directly in the present series of investigations. However, a rough idea of the relationships may be obtained if certain assumptions are made. Data on longitudinal permeability of the same species used in this study were obtained in previous studies by the present writers (*6*). By comparing those values with the values obtained in this work approximation of ratios may be arrived at. The transverse sections were 1 cm. The effective thickness, however, in sections only a few tracheid lengths in thickness, would be less than this if it is assumed for all woods that flow is chiefly through the pit membranes in transverse sections, because the liquid would pass through one-fourth of an open fiber length at each end, on an average (*20*). The number of cell walls traversed would therefore be the actual thickness minus one-half the average fiber length. The average length of jack pine tracheids is about 3.5 mm, depending on the age of the tree and the height. The effective thickness was therefore about 8.3 mm. Let it be assumed that this is the approximate value for the other species also. The effective thickness of the radial and tangential sections is essentially the same as the measured thickness because of the large number of cells and their small diameters. The average thickness for the radial and tangential sections was between 1.25 mm and 1.3 mm. Thus the effective transverse section is about 6.4 times as thick, and this correction must be applied. In doing so it is assumed that the rate of flow varies in inverse proportion to thickness of the section, which does not seem to be strictly true (*23*), but no correction factor is available. The high pressure used in lateral permeability tests was 5 times and 103 times that used for

transverse sections, depending on the kind of wood. In calculating to a common pressure basis, 100 pounds per square inch, a direct proportionality is assumed between rate of flow and applied pressure, which is not always the case. However, in white spruce heartwood and sapwood, black spruce heartwood, and Norway pine sapwood it has been observed that longitudinal rate of flow increased in direct proportion to the pressure applied (*4, 23*). Hence, the pressure correction factor should be eliminated in comparisons on these woods.

On a basis of rate of flow through sections 1 cm thick and at a pressure of 100 pounds per square inch, the relationships in directional permeability of the aforementioned woods are as follows: In white spruce sapwood (all woods referred to were seasoned) the longitudinal permeability was about 70 times the radial permeability and 30,000 times that tangentially; white spruce heartwood was 850 times more permeable longitudinally than radially and 50,000 times more permeable longitudinally than tangentially. In Norway pine sapwood, longitudinal permeability was 2,000 times the radial value and 90,000 times the tangential value. In black spruce heartwood, longitudinal permeability was 110 times the radial permeability and 9,000 times the tangential permeability. In white spruce heartwood, Sutherland, Johnston, and Maass (*23, table VII*) have shown practically an inverse proportionality between longitudinal rate of flow and thickness for sections varying about 250 percent in thickness. If the same may be assumed for increases in radial and tangential thickness, then the values for white spruce heartwood should be valid.

Buckman, Schmitz, and Gortner (*5*) found a disproportionately greater rate of flow with increased pressure in eastern hemlock heartwood. Their data when plotted to a smooth curve show that the rate of flow at 100 pounds per square inch was about five times greater than would be expected if a linear relationship existed with the origin and the flow value at 20 pounds per square inch as the determining points for extrapolation. If this is assumed as a rough means of determining the correction to be applied, then approximations on eastern hemlock directional permeability may be made. The longitudinal permeability thus corrected was about 42,000 times the radial permeability and 10,000 times the tangential permeability.

In the above comparisons, if the longitudinal flow of liquid is assumed to go through the resin canals, where present, then the effective thickness of the transverse section is the same as the actual thickness (1 cm) and the longitudinal values of the ratios are increased by about 12 percent. A second consideration is that even if flow decreases faster with increasing thickness of the section than in simple proportion, then in adjusting for thickness of thin radial and tangential sections to a common basis, 1 cm for example, the corresponding flow values would also be decreased in disproportion and when comparisons are made to longitudinal flow, the directional permeability ratios would be even greater than those reported above.

Somewhat the same argument applies to the pressure adjustments from low to high pressure. The rate-of-flow values obtained at lower pressures and extrapolated linearly to high pressure should be minimum values because actually the rate of flow may be greater than a linear function of pressure, which would result in higher values than those obtained by extrapolation. This would result in greater differences between longitudinal and lateral rates of flow values.

The date of Buckman, Schmitz, and Gortner (*5*) permit the elimination of the pressure variable with a few woods. In certain of their experiments on longitudinal rate of flow, woods were run to equilibrium at a pressure of 100 pounds per square inch. This permits direct comparison from the pressure standpoint and leaves only the possible error in adjusting for greater thickness of sections which is not included in the comparative results. The thickness of the transverse sections in their experiments was from 1.1 to 1.35 cm. The smaller area exposed to the path of flow in their apparatus was corrected for in the rate-of-flow values used for the comparisons to be given.

For the five woods which they used and which were also used in this investigation, the directional permeability comparisons are in reasonably good agreement considering the fact that different samples of wood were used and that the conditions of the experiment were not exactly the same. In the case of balsam fir heartwood the ratios are greater when the observed rate of flow is used than when the value calculated from the rate of flow at 20 pounds per square inch is used and assuming a proportional change in computing the flow at 100 pounds pressure. The same was true with eastern hemlock heartwood. This undoubtedly was due chiefly to the disproportionate increase in rate of flow with increase of pressure. The flow was measured at the desired pressure in the one case and in the other it was computed linearly from a value which was actually on a curve instead of on a straight line. For Norway pine sapwood and white spruce sapwood, however, the use of a linear relationship in computing the flow at high pressure gives fair agreement with their observed values for longitudinal flow. This also confirms reports that in these woods the rate of flow varies in direct proportion to the pressure.

The ratio of longitudinal to radial to tangential permeability is given in table 4 for a number of woods. In certain of these ratios the longitudinal value was calculated by using the data of Buckman, Schmitz, and Gortner (*5*), the rate-of-flow values of which were obtained at 100 pounds per square inch. In the other ratios the longitudinal value was calculated from the data of Erickson, Schmitz, and Gortner (*6*) which was computed by direct proportion to the basis of 100 pounds per square inch. In both methods a simple inverse ratio between permeability and thickness of the section has, of necessity, been assumed.

In table 4 if the radial permeability of springwood and summerwood were measured separately the smaller value was used for comparison. The ratios given are only approximations in round numbers. In most instances the ratios for the sapwoods are considerably greater than those for the heartwoods except in those woods that decrease in permeability from seasoning. The effective thickness of the transverse sections of conifers was considered to be the actual thickness minus 0.17 cm for the sake of uniformity and because it is not definitely known whether longitudinal flow induced by pressure occurs chiefly through resin canals when they are present. In the two hardwoods the actual thickness was used. For reasons pointed out previously, some of the longitudinal values of the ratios are probably too small.

TABLE 4.—*Ratio of longitudinal to radial to tangential permeability for woods of several species*

Kind of wood	Ratio of directional permeabilities			Kind of wood	Ratio of directional permeabilities		
	Longitudinal	Radial	Tangential		Longitudinal	Radial	Tangential
Norway pine sapwood	90,000	40	1	Jack pine sapwood	400,000	150	1
Do	[1] 70,000	40	1	Jack pine heartwood	5	1	0
Norway pine heartwood	50,000	1	0	Longleaf pine sapwood	100,000	150	1
Balsam fir sapwood	200,000	2	1	Longleaf pine heartwood	9,000	1	0
Balsam fir heartwood	120,000	1	1	Black spruce heartwood	10,000	90	1
Do	[1] 900,000	1	1	Western hemlock sapwood	600,000	1	4
Northern white cedar sapwood	10,000	1	2	Western hemlock heartwood	7,000	1	2
Northern white cedar heartwood	60,000	1	10	Slash pine sapwood (based on initial flow)	30,000	45	1
Do	[1] 60,000	1	10	Douglas fir (coast type) sapwood	1×10^7	6,000	1
Eastern hemlock sapwood	100,000	2	1	Douglas fir (coast type) heartwood	50,000	700	1
Eastern hemlock heartwood	9,000	1	4	Paper birch sapwood	1.7×10^6	3	1
Do	[1] 150,000	1	4	White oak sapwood	35,000	7	1
White spruce sapwood	30,000	45	1				
Do	[1] 20,000	45	1				
White spruce heartwood	50,000	60	1				

[1] Longitudinal value of ratio calculated by using data of Buckman, Schmitz, and Gortner (*5*), rate-of-flow values of which were obtained at 100 pounds per square inch.

Because of the variety of conditions under which other data have been obtained, namely, different thicknesses of test specimens, different pressures, pretreatments, temperatures, liquids, and time, it seems futile to attempt critical and detailed comparisons of springwood and summerwood permeabilities and of heartwood and sapwood permeabilities. The conclusion that in most conifers summerwood is more permeable than springwood (whether longitudinal, radial, or tangential is often not specified) is shared by Weiss (*28*), Teesdale (*24*), Scarth (*16*), MacLean (*14*), Bailey (*1*), and Griffin (*7*, *8*) from their investigations on one or more woods. This generalization does not find much support from the data of these experiments on radial permeability. The results of Buckman's experiments (*4*) on creosote distribution in southern pine sapwood appear to be in somewhat better agreement with these data than do those of the above-mentioned experiments. The considerably greater longitudinal permeability of the springwood as compared to the summerwood as found by Erickson, Schmitz, and Gortner (*6*) in two southern pine sapwoods does not seem to hold true in the radial direction. Radially, there was little difference in rate of flow between springwood and summerwood of the southern pine sapwoods. The direction of flow, therefore, appears to affect the relationship of springwood and summerwood permeability.

No information was obtained, by the technique employed, on the relative permeability of springwood and summerwood in the tangential direction. It would seem that there must be radial movement for penetration but that ease of tangential flow would insure better distribution of the fluid if the radial movement tended to be erratic or localized.

The greater radial permeability of the sapwood as compared to that of the heartwood in the resinous woods is in accordance with the results of investigations by Weiss (*28*) and Teesdale (*24*) on creosote penetration. Teesdale found no difference in absorption or penetration between the heartwood and sapwood of eastern and western hem-

lock. In this investigation western hemlock was about as permeable (radially) in the heartwood as in the sapwood, but the heartwoods of eastern hemlock, balsam fir, and northern white cedar were from less than one-tenth to less than one-thousandth as permeable as the corresponding sapwoods. The low value for northern white cedar heartwood may be explained on the basis of the infiltrated substances but there seems to be no satisfactory explanation of the differences in the other species unless it is that the process of heartwood formation affects the permeability of some species more than others.

The sapwoods of white oak and paper birch were both permeable at high pressure. Teesdale and MacLean (*25*) also report that the sapwoods of hardwoods are fairly amenable to preservative treatment even in species in which the heartwood is difficult to treat. White oak is one of this group. Though the ease of preservative treatment may be due somewhat to the open vessels in the sapwood of the hardwoods, this cannot be the case in these investigations because of the method of preparing the sections; hence, the flow obtained was truly radial flow.

During the investigations with black spruce, white spruce, and balsam fir erratic results were occasionally obtained when sections cut from near the border line of heartwood and sapwood were used in permeability tests. A section which was sapwood according to the moisture line gave about the same permeability as the heartwood sections of the same species. The same situation was sometimes true for heartwood sections cut within several rings of the border line as determined by the moisture content in the green wood The border-line zone was therefore avoided in cutting out sections for permeability tests in all species.

It is fully realized that imperfections in the sections, either due to inherent defects in the wood or to improper sawing of the section, would influence the results to a greater or lesser extent. Only wood free from defects such as small knots, compression wood, traumatic canals, etc., was used. In a few woods the grain was so irregular and wavy or the wood rays so curving that perfect sections were practically impossible to obtain from the blocks. This trouble was encountered more often with the radial than with the tangential sections. However, so far as could be discerned by comparisons with other sections, the error involved is not serious, particularly from a qualitative standpoint. It is not denied, however, that it may have made the difference between a measurable flow and no flow at all in a few sections in several woods whose radial permeability was very much greater than their tangential permeability as, for example, Douglas fir.

On an average, there was less variation between sections in tangential flow than in sections on which radial flow was measured. This may be explained thus: In radial flow only a fraction of a ring to several rings, depending on the width of the annual rings, is measured in any one section. Usually it was one ring or less. Hence, the permeability of four annual rings, let us say, would be measured separately. In tangential flow, however, several rings are included in a section because the plane of the section is at right angles to the rings. The flow obtained, therefore, would be the combined flow through several rings. Hence a more average value would be expected than in the case of radial flow. This consideration is exclusive

of the effect that resin canals in resinous woods may have on permeability.

The thickness of the sections for the same or for different species was not always the same but in most sections did not go outside the limits of 1.2 to 1.35 mm and was usually near the average of these limits. No consistent correlation of rate of flow with small differences in thickness was evident. A test run on a set of tangential sections of birch sapwood (which normally seemed to be quite uniform in permeability) of different thicknesses showed that differences of about one-fourth did not make a serious difference in flow in such a small sample. It may be assumed, therefore, that the errors due to differences in thickness of the sections are less than the variations due to sampling even in the same piece of wood.

SUMMARY AND CONCLUSIONS

The radial and tangential permeability to water of 16 species of wood in the seasoned state have been determined. Heartwood, sapwood, springwood, and summerwood were studied when conditions permitted it. Most of the sections were 1.25 to 1.30 mm thick.

Most of the sapwoods of the resinous species were appreciably permeable radially at a pressure of 10 cm of mercury. In general, this was not true of the nonresinous woods.

Nearly all woods, whether heartwood or sapwood, were permeable radially at 100 pounds per square inch. The resinous sapwoods were much more permeable and showed greater variation than the nonresinous sapwoods.

Sapwood was more permeable radially than the heartwood of the same species, except in rare cases. The range of their ratios was very wide.

Summerwood was almost as permeable as springwood in the radial direction at high pressure in the sapwood of the species tested in this way. In the heartwood, springwood permeability was usually greater.

The woods have been grouped on the basis of their magnitude of radial permeability to water at high pressure. A similar arrangement has been made on the basis of tangential permeability of the woods.

The tangential permeability of resinous woods both in the heartwood and in the sapwood was very much less than the radial permeability.

Some heartwoods, including four pines and two hardwoods, were practically impermeable tangentially when judged by the methods employed.

The differences between radial and tangential permeability in nonresinous woods were usually small or of questionable significance.

Several species were about as permeable tangentially in the heartwood as in the sapwood. Other species were definitely more permeable in the sapwood and to a varying degree.

From a consideration of the experimental data and the structural features of wood, the radial flow through the sections of resinous sapwood apparently was chiefly through the resin canals. Radial flow through the resinous heartwood sections may have been assisted by the resin canals or the wood rays, or both.

The uniformity of radial rates of flow of the nonresinous sapwoods, their lower values as compared to the resinous sapwoods, and other considerations suggest that the wood rays are important paths of

radial flow in these sapwoods. Among the heartwoods, the permeabilities were very different, probably due to differences in the transition to heartwood.

In general, the rate of flow decreased with increasing time of continuous flow and approached equilibrium. In some cases a short period of increasing rate of flow preceded the decrease. In rare instances the rate of flow increased somewhat even in the later periods of flow.

Factors such as the surface tension in the capillaries, the void volume of the section, the presence of air in the wood, and the condition of the resin canals may have been responsible singly or collectively for some of the observed variations in flow.

The ratios of the permeabilities in the three structural directions are given for a number of woods. When rate-of-flow values were calculated to a standard basis, longitudinal permeability was usually thousands of times greater than lateral permeability.

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A STREAK DISEASE OF PEAS AND ITS RELATION TO SEVERAL STRAINS OF ALFALFA MOSAIC VIRUS [1]

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INTRODUCTION

A virus disease of peas (*Pisum sativum* L.), manifested by a streaking of the stems and leaves and a spotting of the pods, was observed under greenhouse conditions at the Arlington Experiment Farm, Arlington, Va., in the fall and winter of 1934. The disease in general resembles the streak disease described by Linford,[3] in 1929, as occurring in pea fields throughout the United States, and the spotted wilt of peas recently described by Whipple (*17*).[4] The viruses of alfalfa mosaic likewise produce on peas and other hosts, symptoms that are somewhat similar to those produced by the pea streak virus. In the early stages of these investigations it was reported by Zaumeyer and Wade (*21*) that the pea streak disease was caused by an alfalfa mosaic virus. This assumption was based on the similarity of symptoms produced by the pea streak virus and the alfalfa mosaic viruses on a number of hosts. Since that time it has been proved by Zaumeyer (*18*) that the pea streak virus is different from the alfalfa viruses reported here. It is likely, however, that these viruses may be related. This paper reports the results of a study of three strains of the alfalfa mosaic virus in comparison with the pea streak virus. Data are also presented on the differentiation and identification of these viruses.

HISTORY

The origin of the pea streak disease herein reported is not definitely known. In a proposed study of resistance and susceptibility of peas to a virus of red clover (*Trifolium pratense* L.), a number of pea varieties were planted in greenhouse benches. Windsor broadbeans (*Vicia faba* L.) were planted every fifth row and later were inoculated with a virus from red clover collected at Arlington. After the mosaic symptoms appeared on *V. faba*, large numbers of the pea aphid, *Illinoia pisi* (Kalt.), which had been reared on healthy *V. faba* plants for several weeks in cages, were released in the greenhouse by being placed on *V. faba* plants that were growing in the greenhouse benches and were infected with the red clover mosaic virus. The aphids later migrated or were artificially transferred to the peas growing in adjacent rows. The pea aphids used were originally collected from apparently healthy alfalfa plants growing in the field near Arlington.

About 3 weeks after the release of the aphids, spots resembling

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[3] LINFORD, MAURICE B. PEA DISEASES IN THE UNITED STATES IN 1928. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 67, 14 pp. 1929. [Mimeographed.]

[4] Reference is made by number (italic) to Literature Cited, p. 771.

those produced by *Alternaria* sp. appeared on the leaves of the *Vicia faba* plants. Microscopic and cultural studies proved that these lesions were not produced by fungi or bacteria. These spots were followed by a streaking of the stems and petioles, defoliation, and finally by the death of many plants. Somewhat similar symptoms appeared on the peas, and within a short period the disease became widespread throughout the peas and Windsor broadbeans growing in the greenhouse.

After inoculation with the virus from red clover and before release of the aphids to them, only the typical mottling appeared on the inoculated *Vicia faba* plants. Since the streak disease first appeared on the *V. faba* plants about 3 weeks after the release of the aphids, it was assumed that the virus may have originated from infective aphids. Freezing outdoor temperatures at that time made it impossible to determine whether the alfalfa plants where the aphids were collected were the source of the disease. In the spring of 1935, however, it was found that a high percentage of the alfalfa plants in the field were infected with mosaic.

Collections of mosaic-infected alfalfa plants were made both at Arlington, Va., and in Colorado. Inoculations with the viruses of the mosaic-infected plants showed that two viruses were present in the material from Virginia and that both were different from the virus collected in Colorado. The three viruses, when inoculated to *Vicia faba*, produced symptoms somewhat similar to the pea streak virus. On peas the symptoms of one of the alfalfa viruses showed marked similarities to the pea streak virus.

Notwithstanding this similarity in symptoms, the pea streak virus differs distinctly from the alfalfa viruses. Since it has been impossible to isolate the pea streak virus from later collections of mosaic-infected clover material, it is believed that the virus may have originated from infected alfalfa plants and may have been transmitted to peas and Windsor broadbeans by the pea aphid.

EARLIER INVESTIGATIONS

Linford,[5] in 1928, described a streak disease of peas that he found in pea fields from the Atlantic coast west to Utah and Montana. He (*5*) later found that *Thrips tabaci* Lind., if transferred to peas after having fed on infected *Emilia sagittata* (Vahl) DC., produced symptoms identical with the earlier recorded pea streak. Likewise thrips reared on infected pea plants transmitted the disease to peas as well as to pineapple, producing the typical yellow spot disease.

Weimer (*15, 16*) reported on the transmissibility of an alfalfa mosaic but recorded no cross-inoculation studies. Zaumeyer and Wade (*19, 20*) showed that the alfalfa virus of Weimer, as well as an alfalfa virus collected in Virginia, was infectious to beans and other legumes. Pierce (*8*) also described an alfalfa mosaic that was infectious to bean, pea, *Vicia faba*, and other leguminous hosts.

Zaumeyer and Wade (*19, 22*) and later Pierce (*7*) showed that the viruses of sweetclover and white clover cause streak symptoms on pea.

Pierce (*7*) also reported a broadbean local-lesion virus that produced streak symptoms on peas, especially when in combination with other viruses. The relation of this virus to Linford's pea streak virus was not shown.

[5] LINFORD, MAURICE B. See footnote 3.

Whipple (*17*) recently reported a pea streak as being caused by the spotted wilt virus. His investigations showed that this disease was very similar to Linford's pea streak and pineapple yellow spot virus (*5*, *6*).

Snyder and Thomas (*12*) showed that the spotted wilt virus was infectious to sweet peas and produced a streaking of the stems. Stubbs (*13*) produced a similar symptom on garden peas with the tobacco ring spot virus.

MATERIALS AND METHODS

VIRUSES

Pea streak virus 1—This virus was obtained from garden pea plants grown at Arlington, Va., in 1934. As mentioned previously, the disease appeared after pea aphids collected from alfalfa plants growing under field conditions were released in the greenhouse. This virus, when inoculated into pea and *Vicia faba*, produced a decided streaking of the stem, petioles, and leaf veins. Since the pea streak virus described by Linford does not appear to be a characteristic legume virus, the virus described in this paper is designated as pea streak virus 1.

Alfalfa mosaic virus 1—Viruses from three alfalfa mosaics were used in these studies; two were collected from mosaic-infected plants grown at Arlington, Va., and one from northeastern Colorado. One of the viruses secured in Virginia was apparently identical with the alfalfa virus described by Weimer (*16*), Pierce (*8*), and Zaumeyer and Wade (*20*). Pierce (*8*) was of the opinion that the alfalfa virus described by Weimer (*16*) and designated as alfalfa virus 1 was not identical with the virus described by him from the same host, which he listed as alfalfa virus 2. Subsequent studies by the writer suggest that these two viruses are identical. This virus will be discussed herein as alfalfa mosaic virus 1.

Alfalfa mosaic virus 1A.—The alfalfa virus collected from northeastern Colorado reacted similarly to alfalfa mosaic virus 1. Its specificity was established by the fact that it produced severer symptoms on peas and on *Vicia faba* than alfalfa mosaic virus 1, and by differences in host range and properties. The evidence presented in this paper indicates that this virus is a strain of alfalfa mosaic virus 1; it is designated here as alfalfa mosaic virus 1A.

Alfalfa mosaic virus 1B.—The third alfalfa mosaic virus was separated from material containing alfalfa mosaic virus 1. When a large number of pea plants of the Perfection variety were inoculated with alfalfa mosaic virus 1 all the plants except one manifested the typical mild mottling produced by alfalfa mosaic virus 1. This plant showed decidedly more extreme symptoms than the others, indicating a virus distinct from alfalfa mosaic virus 1. Symptomatology, host range, and properties in vitro showed the virus to be different from both alfalfa mosaic viruses 1 and 1A. This third virus is designated as alfalfa mosaic virus 1B.

METHODS

The experimental work was conducted in the greenhouse at approximately 14° to 21° C. when peas were inoculated and at 20° to 25° when beans were inoculated. The inoculum was secured from either

young infected peas or *Vicia faba* plants. Viruses from both hosts gave identical results. The virus extracts were prepared in the usual manner, by macerating diseased tissue in a sterile mortar and straining the juice. The solid residue was placed in cheesecloth and used as an inoculating pad. Leaflets of young plants were dusted with carborundum powder and rubbed with the pad saturated in the expressed mosaic-infected juice.

The methods used in the studies on thermal inactivation and on dilution were similar to those described by Johnson and Grant (*4*). The aging tests were made by storing the juice of infected plants in stoppered flasks in a darkened, constant-temperature chamber at 18° C. Inoculations were made at the desired intervals in the usual manner.

HOSTS

The principal hosts used in the differential studies of the viruses were garden peas, *Vicia faba*, and beans. Since certain pea varieties show more extreme symptoms than others when inoculated with the several viruses, a few were selected as test varieties. These were a variety known as Capucijner, which is an edible-pod pea grown widely in the Netherlands; Mammoth Melting Sugar, another edible-pod variety; and Dwarf Telephone. The bean varieties used were Stringless Green Refugee, Corbett Refugee, Robust, and Great Northern U. I. No. 1. Other leguminous hosts used are mentioned in a later section.

EXPERIMENTAL RESULTS

The four viruses discussed in this paper are differentiated by (1) the symptoms they produce on peas and other hosts, (2) varietal differences in susceptibility of peas and beans, (3) host range, and (4) certain properties of the viruses in vitro.

SYMPTOMS

Pea Streak Virus 1 on Pea

The first symptom produced on pea plants by pea streak virus 1 is a slight purpling and streaking of the stem and the lower portion of the stipule (fig. 1, *E*). Later the stipules recurve, the leaflets tend to curl downward and to become distorted and twisted, and the veins are decidedly pronounced (*B*, *F*, *H*). The tendrils become gnarled and twisted. Mottling does not occur on the young leaves, but frequently a clearing of the veins and a slight chlorosis is noticeable. The internodes in the apical portion of the plant are shortened and the leaves, which are smaller than usual, do not unfold normally but show a rosetted condition (*D*). The streaking of the stems becomes more intense and later takes on a brownish discoloration, which may extend from the top to the bottom of the plant. The phloem tissue becomes necrotic and discolored. This discoloration may also involve the petioles and main veins of the leaflets. The leaves finally become flaccid, the tip of the plant begins to wilt (*I*), and the plant ultimately dies. It is believed that death is due not to the virus alone but to soil fungi that attack the plant after it becomes weakened by the virus. In some cases only the tip dies, the rest of the plant remaining chlorotic (*I*).

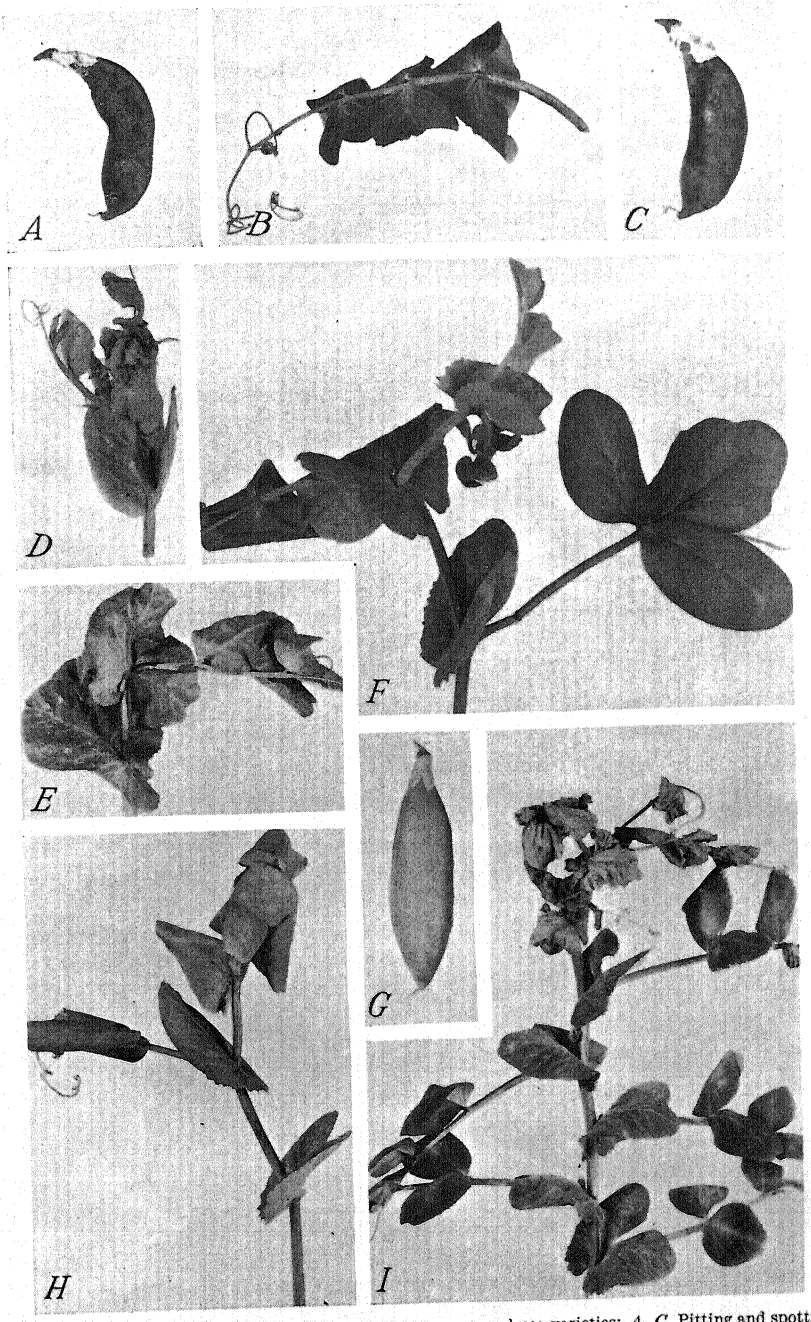


FIGURE 1.—Symptoms produced by pea streak virus 1 on several pea varieties: *A*, *C*, Pitting and spotting of World Record pea pods; *B*, *E*, downward curling of leaflets and streaking of petioles of Green Giant; *D*, terminal rosette and leaf malformation of Green Giant; *F*, infected Capucijner; *G*, healthy pod of World Record; *H*, stem streak and leaf curl of Capucijner; *I*, terminal killing of Admiral.

It frequently happens that a plant infected when young dies before attaining much growth. If it does not die, it is usually decidedly stunted, the leaves become curled and twisted and the veins very pronounced. Such plants are generally very brittle and when removed from the soil do not wilt as readily as do healthy plants.

Infected plants sometimes produce axillary buds, which usually die before much growth develops. When the virus is spread by aphids the symptoms first appear near the apical portion of the plant, but when the plants are artificially inoculated the symptoms are noted just above the point of inoculation.

The pods that are formed before the plant becomes seriously infected take on a dark purplish-gray or brown color over a considerable portion of their surface, but especially along the region of the dorsal suture. They may also be spotted, pitted, and decidedly malformed, and frequently do not reach maturity (fig. 1, *A* and *C*). Seeds from infected pods generally are small and shriveled.

Alfalfa Mosaic Viruses on Alfalfa

The symptoms of alfalfa mosaic viruses 1, 1A, and 1B on alfalfa are identical (fig. 2, *B*). The mosaic is first noted as small, light-greenish or light-yellow areas between or along the veins. Later these chlorotic regions enlarge and become increasingly more yellow in color. Distortion of infected leaves is not uncommon. At high temperatures the symptoms are commonly masked and diseased plants appear normal. The symptoms are most pronounced in the spring and early fall.

Alfalfa Mosaic Virus 1 on Pea

The symptoms produced on pea plants by alfalfa mosaic virus 1 appear in about 8 days after inoculation at 70° to 75° F. At lower temperatures that are more favorable for pea growth they do not develop so quickly and may require 10 to 15 days.

The first apparent symptom is a slight mottling that starts as small, chlorotic areas on the leaves above the point of inoculation. On some varieties these chlorotic areas may be very indistinct, while on others they may be more clearly defined. This mild chlorosis gradually becomes a mild mottling, light-green areas without pattern being found throughout the leaf. The leaves are not yellowed, the stems are not discolored as with pea streak virus 1, and the plants are not stunted. In general the symptoms produced by this virus are very mild on peas (fig. 2, *G* and *J*).

Alfalfa Mosaic Virus 1A on Pea

The symptoms of alfalfa mosaic virus 1A on peas appear in about the same length of time as those produced by alfalfa mosaic virus 1 but are much more pronounced than the latter. In addition to the mottling, which is quite pronounced (fig. 2, *I* and *L*), considerable leaf necrosis occurs. Infected leaves are slightly bronzed and frequently die. The stems appear purple but less strikingly so than in plants infected with pea streak virus 1. On the Dwarf Telephone variety, the symptoms of alfalfa mosaic virus 1A are very striking. The stunting of infected plants is possibly the most conspicuous symptom. The leaves are crinkled, the internodes are decidedly shortened, and

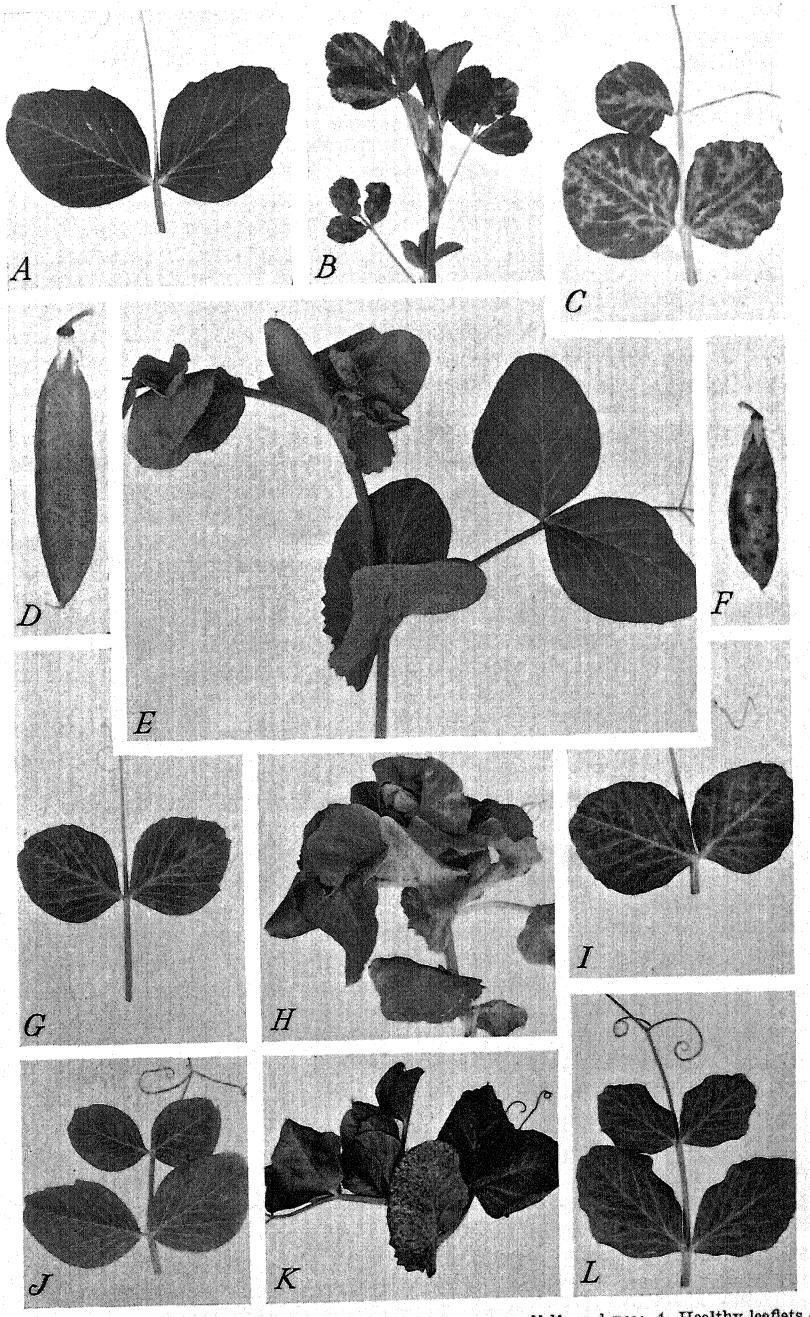


FIGURE 2.– Symptoms produced by the alfalfa mosaic viruses on alfalfa and pea: *A*, Healthy leaflets of Capucijner pea; *B*, mosaic-infected alfalfa; *C*, *E*, *K*, symptom variations produced by alfalfa mosaic virus 1B on Capucijner pea; *D*, healthy pod of World Record pea; *F*, pod of World Record pea infected with alfalfa mosaic virus 1A; *G*, *J*, leaflets of Capucijner and Green Giant pea, respectively, infected with alfalfa mosaic virus 1; *H*, Dwarf Telephone pea infected with alfalfa mosaic virus 1B; *I*, *L*, Capucijner and Green Giant pea, respectively, infected with alfalfa mosaic virus 1A.

the stem is discolored. On the under side of the leaves the veins and veinlets are brownish and appear water-soaked. The brownish color is also noticeable on the upper side of the leaflets, more or less along the veins and veinlets. A purplish discoloration occurs at the base of the leaflets and stipules.

A plant infected when young becomes very bunchy and never grows to more than one-fourth normal size. The uppermost leaves curl or roll inward, and are wavy along the edges, crinkled, and slightly chlorotic. In some cases, the midvein of older leaves is depressed, with an arching of the lamina. The vascular system of infected plants is necrotic.

Infected pods in some cases show a slight to severe dark necrotic discoloration. They are frequently spotted, pitted, and malformed (fig. 2, *F*).

Alfalfa Mosaic Virus 1B on Pea

The symptoms of alfalfa mosaic virus 1B on peas appear in about 8 days, the same length of time after inoculation as those produced by alfalfa mosaic viruses 1 and 1A. The inoculated leaves of susceptible varieties frequently die. Spotting of the remaining leaves follows, the spots usually appearing at the second node above the point of inoculation. Small brown spots appear first on the under side of the stipules and leaflets (fig. 2, *K*), but later are quite pronounced on the entire upper side of the leaflets. Later these spots become necrotic, and an infected stipule or leaflet may be killed. As in plants infected with pea streak virus 1, the stipules recurve and the leaflets tend to curl downward and become distorted (*E*). The stems become streaked (*E*), but the streaks are short, neither so continuous nor so intense in color as those produced by pea streak virus 1. When the infection is serious, alfalfa mosaic virus 1B stunts the plant to about the same degree as does pea streak virus 1. The symptoms produced by alfalfa mosaic virus 1B differ from those by pea streak virus 1, in (1) a pronounced spotting of the leaves and petioles, (2) less streaking of the stem and petioles, and (3) less downward curling and malformation of the leaflets.

In addition to the foregoing symptoms, the leaves of certain varieties are mottled quite intensely with large light-green areas between the veins (fig. 2, *C*). Such leaves are frequently crinkled and malformed. In general, the mottling is more intense on plants infected with alfalfa mosaic virus 1B than on those infected with alfalfa mosaic viruses 1 and 1A. On Dwarf Telephone peas, alfalfa mosaic virus 1B does not produce such pronounced symptoms and does not stunt the plants so much as does alfalfa mosaic virus 1A.

RESISTANCE AND SUSCEPTIBILITY OF HOSTS

Peas

A relatively small number of pea varieties were inoculated with the viruses herein reported except in the case of pea streak virus 1, with which more than 40 varieties were tested. This paper records the results from only those varieties that were tested with the four viruses, the object being to show differences in the reaction of the several viruses as a means of differentiating them.

Seventeen varieties of peas were inoculated with the four viruses under greenhouse conditions (table 1). The seeds were planted in

benches, and the plants were inoculated in the usual manner, carborundum powder being used on the surface of the leaves, which were then rubbed with a cheesecloth pad saturated with an undiluted extract from plants infected with the several viruses.

Table 1 shows that all varieties were susceptible to pea streak virus 1. Thus far no variety tested, including many not reported herein, has shown immunity to this virus. In most cases 100 percent of the plants were infected, although a number of varieties showed moderate symptoms. Alderman and Green Admiral alone manifested mild symptoms.

Alfalfa mosaic virus 1 caused mild symptoms on all varieties except Horal, which was immune. The symptoms on Little Marvel were slightly more pronounced than those on any of the other varieties. Although a high percentage of the plants became infected, the percentage was not quite so high as with pea streak virus 1.

Alfalfa mosaic virus 1A likewise infected all varieties except Horal. The infection in general was more severe with this virus than with alfalfa mosaic virus 1. Of the 16 susceptible varieties, 3 showed severe, 8 moderate, and 5 mild symptoms. Three varieties, Alderman, Green Admiral, and Surprise, in addition to manifesting mild symptoms, showed a lower percentage of infection than any of the others.

TABLE 1.—*Susceptibility and resistance of several pea varieties to pea streak virus 1 and alfalfa mosaic viruses 1, 1A, and 1B*

Variety	Results of inoculation with—											
	Pea streak virus 1			Alfalfa mosaic virus								
				1			1A			1B		
	Plants inoculated	Plants infected	Extent of infection[1]	Plants inoculated	Plants infected	Extent of infection[1]	Plants inoculated	Plants infected	Extent of infection[1]	Plants inoculated	Plants infected	Extent of infection[1]
	Number	*Number*		*Number*	*Number*		*Number*	*Number*		*Number*	*Number*	
Alaska	12	12	a	26	26	c	10	10	a	12	12	a
Alderman	16	11	c	12	10	c	13	5	c	14	14	b
Black Eye Marrow	9	9	a	10	9	c	9	9	b	12	12	a
Capucijner	20	20	a	13	13	c	9	7	c	10	10	a
Dwarf Telephone	12	12	a	47	41	c	36	36	a	10	10	b
Green Admiral	22	22	c	17	14	c	10	3	c	11	11	b
Green Giant	22	22	a	12	10	c	19	19	b	10	10	a
Harrison Glory	20	20	a	22	17	c	36	36	b	10	7	a
Horal	23	23	a	14	0	------	13	0	------	19	0	------
Laxton Progress	10	9	a	10	10	c	14	14	a	13	12	c
Little Marvel	10	10	a	10	9	b	20	18	c	26	26	b
Mammoth Melting Sugar	25	25	a	28	28	c	15	15	b	15	15	b
Perfection	31	31	a	28	28	c	12	12	b	19	19	a
Surprise	15	11	b	12	11	c	16	4	c	11	11	b
White Eye Marrow	21	19	b	19	12	c	10	10	b	10	10	a
Wisconsin Early Sweet	14	14	b	12	12	c	12	12	b	14	14	a
World Record	10	10	b	28	18	c	10	10	b	13	13	a

[1] a, serious infection; b, moderate infection; c, mild infection.

Horal was the only variety not susceptible to alfalfa mosaic virus 1B. In this respect alfalfa mosaic virus 1B reacts in the same way as alfalfa mosaic viruses 1 and 1A. The percentage of plants infected by alfalfa mosaic virus 1B was about equal to that infected by alfalfa

mosaic virus 1, but greater than that infected by alfalfa mosaic virus 1A. The symptoms produced on most varieties of peas by alfalfa mosaic virus 1B were more severe than those produced by the other two strains of alfalfa mosaic virus. Nine varieties showed severe symptoms, six moderate symptoms, and only one, Laxton Progress, mild symptoms.

Although in general the symptoms produced by alfalfa mosaic virus 1B were severer than those produced by alfalfa mosaic viruses 1 and 1A, there were a few exceptions. These seem to be significant in that they afford further aid in distinguishing the alfalfa mosaic viruses.

It was pointed out elsewhere that the Dwarf Telephone variety exhibited the severest symptoms when inoculated with alfalfa mosaic virus 1A; these are a decided stunting of the plant and an intense mottling followed by a crinkling of the leaves. Alfalfa mosaic virus 1 produces a very mild mottling and no stunting; in these respects alfalfa mosaic virus 1B is similar to alfalfa mosaic virus 1, but in addition the former causes a leaf spotting and a slight streaking of the stems.

On Laxton Progress, alfalfa mosaic virus 1A produced the severest leaf mottling, while alfalfa mosaic viruses 1 and 1B produced a mild mottling of about equal intensity.

On Little Marvel, alfalfa mosaic virus 1B produced the strongest leaf mottling of any of the viruses, followed next by alfalfa mosaic virus 1; alfalfa mosaic virus 1A produced the mildest mottling. The last mentioned virus, however, stunted the plants more severely than did the other two.

Beans

The bean varieties tested with the four viruses were Stringless Green Refugee, Corbett Refugee, Robust, and Great Northern U. I. No. 1 (table 2). All of these varieties, except Stringless Green Refugee, are resistant to the common bean mosaic virus. Twenty plants of each variety were inoculated with the pea streak virus and three lots, of 10 plants each, of each variety were inoculated with the three alfalfa mosaic viruses, respectively. The inoculations were made by lightly rubbing the leaves with an undiluted extract of the viruses. Carborundum powder was applied only with the pea streak virus. Since the alfalfa mosaic viruses produce local necrotic lesions on beans (fig. 3, *D* and *E*), the lesions on 20 inoculated leaves were counted to determine the degree of susceptibility. Where the lesions were very numerous, as in the case of Stringless Green Refugee, the number was estimated.

None of the four varieties was infected by pea streak virus 1. Alfalfa mosaic viruses 1, 1A, and 1B were infectious to all of the varieties (table 2). Stringless Green Refugee was the most susceptible to the three alfalfa mosaic viruses, as determined by the total number of local lesions produced; Great Northern U. I. No. 1 was next in susceptibility; Corbett Refugee and Robust showed about the same reaction. Alfalfa mosaic virus 1B produced fewer lesions on Stringless Green Refugee but a greater number of lesions on the other three varieties than did alfalfa mosaic virus 1A. Alfalfa mosaic virus 1 produced the smallest number of lesions on all varieties tested.

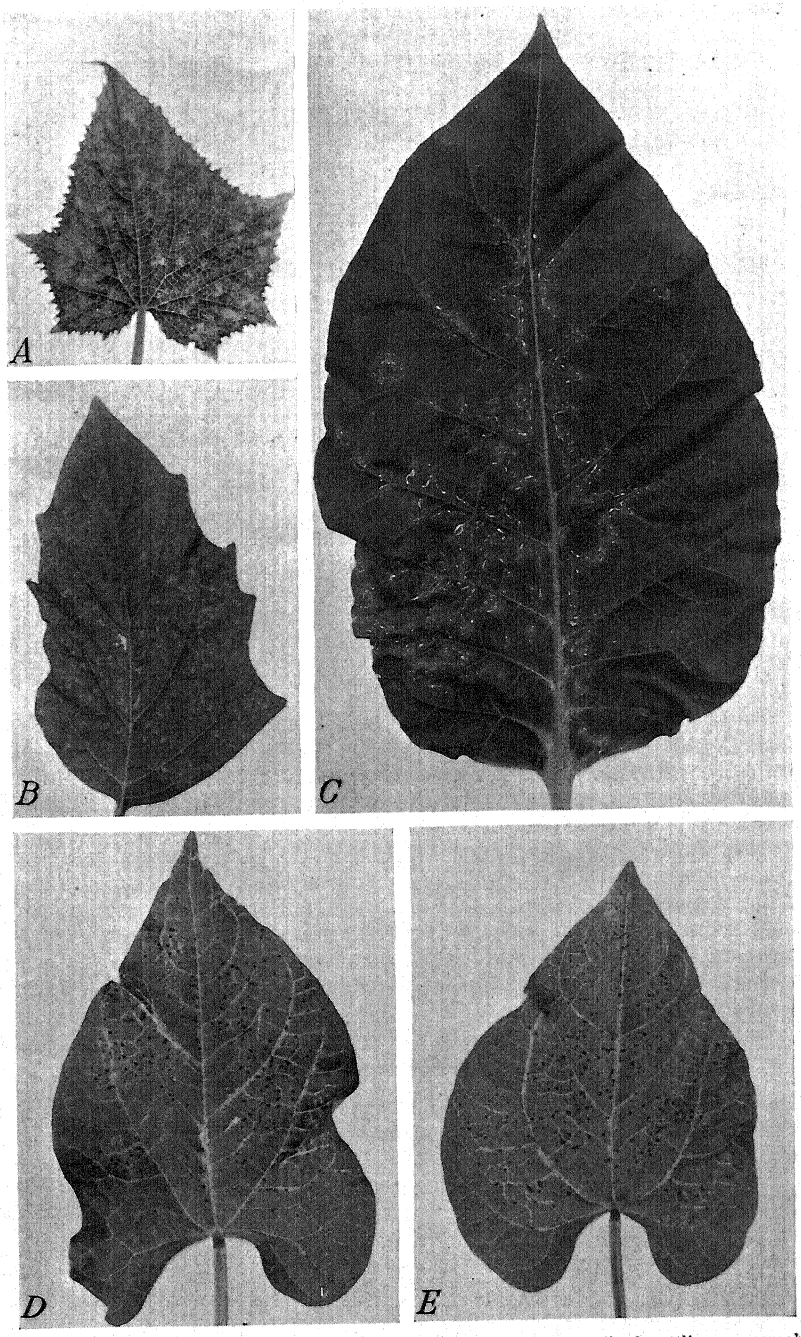


FIGURE 3.—Symptoms produced by alfalfa mosaic viruses on several hosts: *A*, Leaf mottling on cucumber produced by alfalfa mosaic virus 1A; *B*, mottling on *Datura stramonium* infected with alfalfa mosaic virus 1B; *C*, leaf pattern produced by alfalfa mosaic virus 1B on Turkish tobacco; *D*, *E*, local lesions produced by alfalfa mosaic viruses 1 and 1A, respectively, on Stringless Green Refugee bean; note higher percentage of light-tan centers of lesions in *E* than *D*.

TABLE 2.—*Susceptibility and resistance of four bean varieties to pea streak virus 1 and alfalfa mosaic viruses 1, 1A, and 1B*

Variety	Reaction[1] to—							
	Pea streak virus 1		Alfalfa mosaic virus					
			1		1A		1B	
	Plants inoculated	Plants infected	Plants inoculated	Total lesions[1]	Plants inoculated	Total lesions[1]	Plants inoculated	Total lesions[1]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Stringless Green Refugee	20	0	10	1,540	10	8,500	10	3,700
Corbett Refugee	20	0	10	26	10	82	10	235
Great Northern U. I. No. 1	20	0	10	35	10	237	10	506
Robust	20	0	10	29	10	71	10	258

[1] Total number of lesions produced on 20 inoculated primary leaves.

The size of the lesion varies, depending on the virus and the variety inoculated. On Stringless Green Refugee alfalfa mosaic virus 1 produced lesions from 1μ to 1½μ in diameter. The lesions produced by alfalfa mosaic viruses 1A and 1B on this variety averaged about 2μ in diameter. On Corbett Refugee the lesions were slightly smaller than on Stringless Green Refugee. On Robust and Great Northern U. I. No. 1, the lesions produced by alfalfa mosaic virus 1 were from 2μ to 2½μ in diameter, while those produced by alfalfa mosaic viruses A1 and 1B averaged approximately 2½μ to 3μ in diameter.

In addition to the production of local necrotic lesions, the alfalfa mosaic viruses frequently followed the veins and veinlets, causing them to become necrotic. This infection did not extend beyond the inoculated leaves. Alfalfa mosaic virus 1 produced only a slight vein necrosis on the inoculated leaves of Stringless Green Refugee, Corbett Refugee, and Great Northern U. I. No. 1. On Robust more vein necrosis was noted than on the other varieties.

Alfalfa mosaic viruses 1A and 1B produced more vein necrosis than alfalfa mosaic virus 1. On the Robust variety the former two viruses produced this type of infection more severely than on the other varieties. The necrosis frequently extended for a distance of 8μ to 10μ along veins and veinlets.

OTHER HOSTS

In order to determine the host range of the four viruses, a number of species in the genus *Phaseolus*, as well as species in other genera, were inoculated with the pea streak virus and with alfalfa mosaic viruses 1, 1A, and 1B, with results as shown in table 3.

It is evident from table 3 that the host range of pea streak virus 1 is more limited than that of the three strains of alfalfa mosaic virus. The following hosts were susceptible to pea streak virus 1: *Cicer arietinum, Lens esculenta, Medicago sativa, Melilotus alba, Soja max, Trifolium incarnatum, T. pratense, T. repens, Vicia faba, V. faba minor*, and *V. sativa*. In addition, alfalfa mosaic virus 1 was infectious to *Lupinus albus, L. luteus, Phaseolus angularis, P. aureus, P. lunatus, P. mungo, Vigna sesquipedalis, V. villosa, V. sinensis, Nicotiana tabacum, Petunia hybrida*, and *Datura stramonium*. This host range is more ex-

tended than that reported earlier for the same virus by Zaumeyer and Wade (*20*). It corresponds in general with that of Pierce (*8*), who tested a number of plant species with an alfalfa mosaic virus.

TABLE 3.—*Susceptibility and resistance of various legumes and other plants to pea streak virus 1 and alfalfa mosaic viruses 1, 1A, and 1B*

Plant	Reaction [1] to—							
	Pea streak virus 1		Alfalfa mosaic virus					
			1		1A		1B	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Cajanus indicus Spreng. (pigeonpea)	7	0	8	0	5	0	10	0
Cicer arietinum L. (chickpea)	5	5	10	9D	10	8D	10	10D
Lathyrus odoratus L. (sweet pea, Pink Cockade variety)	10	0	15	0	20	8	15	0
Lens esculenta Moench (lentil)	16	10	20	18D	20	20D	20	20D
Lespedeza striata (Thunb.) Hook. and Arn. (lespedeza)	5	0	5	0	5	1	5	2
Lupinus albus L. (white lupine)	6	0	15	15L	18	18L	10	10L
L. luteus L. (lupine)	10	0	10	10	5	5	10	9
Medicago sativa L. (alfalfa)	5	2	5	1	5	3	5	2
Melilotus alba Desr. (white sweetclover)	5	4D	5	1D	5	1D	5	4D
Phaseolus acutifolius latifolius Freeman (tepary bean)	9	0	15	0	15	0	15	0
P. angularis (Willd.) W. F. Wight (adzuki bean)	11	0	10	8	10	10	10	10
P. aureus Roxb. (mung bean)	12	0	10	10L	15	15L	12	12L
P. calcaratus Roxb. (rice bean)	6	0	15	0	17	0	12	0
P. lunatus macrocarpus Benth. (lima bean)	5	0	5	0	5	0	10	0
P. lunatus L. (sieva bean, Henderson Bush variety)	5	0	10	9	12	12	10	10
P. mungo L. (urd bean)	20	0	9	2L	21	17	12	8L
Soja max (L.) Piper (soybean)	10	10	10	10	15	14	10	10
Stizolobium deeringianum Bort. (velvetbean)	5	0	10	0	10	0	9	0
Trifolium incarnatum L. (crimson clover)	5	4	5	5	5	3	5	5
T. pratense L. (red clover)	5	2	5	1	5	4	5	4
T. repens L. (white clover)	5	3	5	1	5	1		
Vicia faba L. (broadbean)	10	10 L	10	10 L	10	10 L	10	10 L
V. faba minor (small-seeded broadbean)	10	10 L	10	10 L	9	9 L	12	12 L
V. sativa L. (spring vetch)	16	11 L	10	8 L	10	8 L	12	10 L
V. villosa L. (vetch)	20	0	20	16 L	20	18 L	18	18 L
Vigna sesquipedalis (L.) Fruwirth (asparagus-bean)	12	0	10	10 L	10	10 L	10	10 L
V. sinensis (L.) Endl. (cowpea)	7	0	10	10 L	10	10 L	10	10 L
Nicotiana tabacum L. (Turkish tobacco)	5	0	5	5 L	5	5 L	5	5 L
Petunia hybrida Vilm. (petunia)	5	0	5	5	5	5	5	5
Datura stramonium L. (jimsonweed)	5	0	5	5	5	5	5	5
Lycopersicon esculentum Mill. (tomato)	5	0	5	0	5	0	5	0
Solanum tuberosum L. (potato)	5	0						
S. melongela L. (eggplant)	5	0	5	0	5	0	5	0
Capsicum annuum L. (pepper)	5	0						
Zinnia elegans Jacq. (zinnia)	5	0	10	0	13	1	10	4
Cucumis sativus L. (cucumber)	5	0	10	0	10	8	10	8

[1] D represents death of plants; L, local lesions.

Alfalfa mosaic virus 1A was infectious to the hosts just mentioned and in addition to *Cucumis sativus, Lathyrus odoratus, Lespedeza striata* and *Zinnia elegans*. Alfalfa mosaic virus 1B infected all the hosts that were susceptible to alfalfa mosaic virus 1A except *Lathyrus odoratus*.

SYMPTOMS OF THE VIRUSES ON DIFFERENTIAL HOSTS

It is not intended in this paper to describe in detail the symptoms produced by the several viruses on all the hosts that were inoculated with the four viruses. Certain distinct differences were noted on a number of hosts, both as to type of infection and severity of symptoms produced. It is believed that these differences are helpful in the differentiation and identification of the viruses.

On *Vicia faba* pea streak virus 1 produces very characteristic symptoms. The first symptom that appears is a local ring spot lesion, 3 to 5 mm in diameter, on the inoculated leaves (fig. 4, *C* and E), followed by a slight red to brown streaking of the stems (*A*). The stem streak progresses and may extend from the ground line to the uppermost tip of the plant, covering in some cases almost the entire stem (*F*). The petioles may also be streaked (*D*), as well as the veinlets on the under side of the leaflets.

On certain of the lower leaves of *Vicia faba* plants infected with pea streak virus, the circular ring spot lesions may cover a part or the whole of a leaflet (fig. 4, *C* and *E*). In other cases circular zonate spots appear which may coalesce with others, producing large dark-gray to black spots resembling the lesions produced by *Alternaria* sp. These areas are surrounded by a yellowish border and may appear in the center of the leaflet, but most frequently they are found along the margins of the leaf. If the lesions are large, the infected leaflet may wither and die (*F*).

The tips of *Vicia faba* plants infected with pea streak virus 1 are often rosetted. The leaves are smaller than normal, do not unfold properly, and are spotted and streaked (fig. 4, *B*). The plants may gradually die, beginning at the growing point, and in such cases the stem may show only a small amount of streaking (*B*). In other cases the entire stem may be reddened, normal-appearing leaflets being found below the dead tissue (*F*).

Alfalfa mosaic virus 1 also produces local, necrotic, reddish to brownish lesions on *Vicia faba*. The inoculated leaves later die. The uppermost leaves curl inward and show a stippled bronzing that later becomes a more distinct, reddish spotting. The spots are usually more numerous at the base of the leaflet (fig. 5, *E*). These leaves frequently die while some of the lower leaves still appear normal. The stem may be shrunken and decidedly streaked, the streaks being blackish brown. Seriously infected plants usually die.

The symptoms produced by alfalfa mosaic viruses 1A and 1B on *Vicia faba* are essentially the same as those produced by alfalfa mosaic virus 1, except that they are more severe. Alfalfa virus 1B produces the most striking symptoms. The local necrotic lesions produced by this virus on the inoculated leaves are larger and more numerous than those produced by either of the other alfalfa mosaic viruses (fig. 5, *B*). These lesions may cover an entire leaflet, causing it to die 5 days after the appearance of symptoms. The tip of the plant (*C*) frequently shows the effect of infection shortly after the local lesions appear on the inoculated leaves (*B*), and often it is killed 10 to 12 days after inoculation. The stem then begins to shrink, takes on a black discoloration (*C*), and becomes internally necrotic.

As already mentioned, the pea streak virus is not infectious to bean, whereas the three alfalfa mosaic viruses produce local necrotic lesions on the inoculated leaves (fig. 3, *D* and *E*). The only differ-

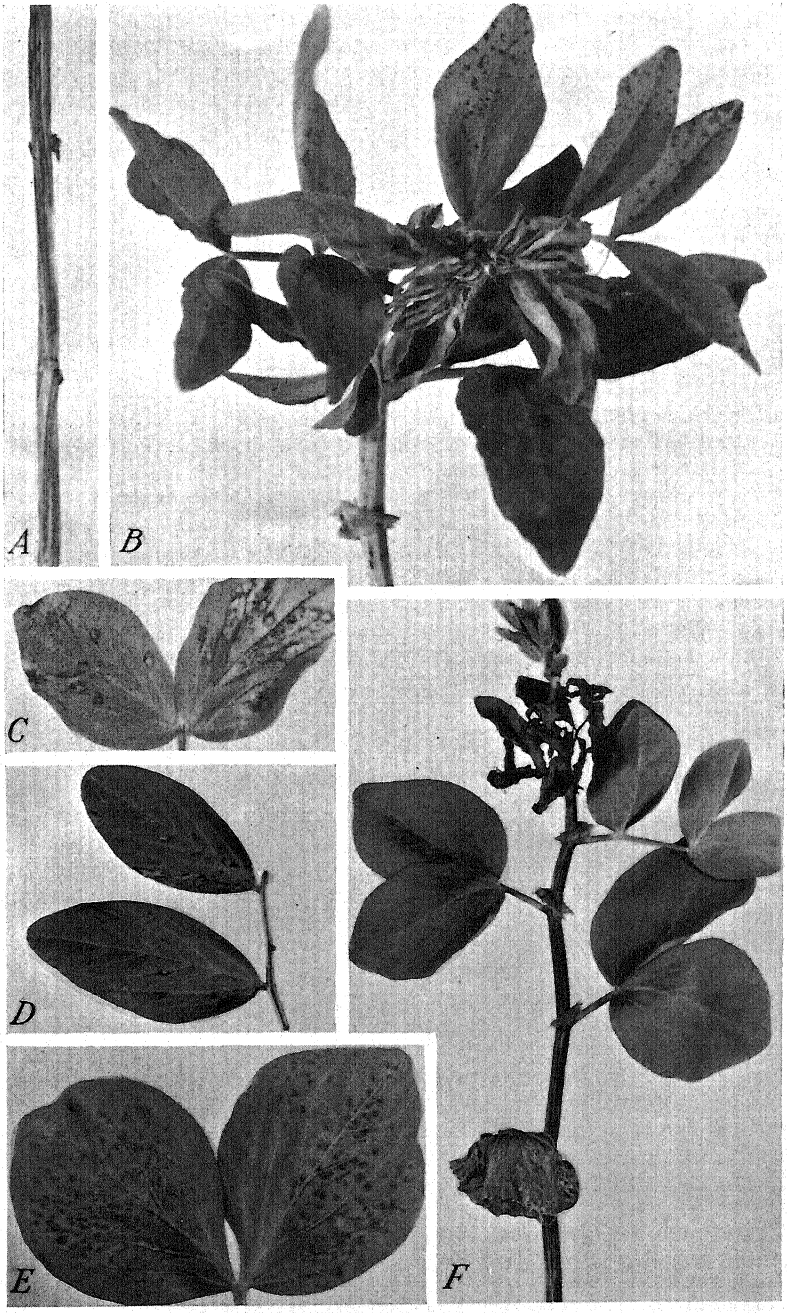


FIGURE 4.—Symptoms produced by pea streak virus 1 on Windsor broadbean: *A*, streaking of stem; *B*, leaf spot and rosette; *C*, *D*, *E*, ring-spot-like symptoms on leaflets; *F*, stem streaking and killing of inoculated and terminal leaflets; center leaflets appear normal.

ences between the symptoms produced by the latter viruses on bean were in the size of the lesions produced and in the formation of light-tan centers. The local lesions produced by alfalfa mosaic virus 1 never exceeded 1 to 2½ mm in diameter and were brownish red, with only an occasional light-tan center (*D*). Alfalfa mosaic viruses 1A (*E*) and 1B produced lesions 2 to 3 mm in diameter with a high percentage of light-tan centers.

On white sweetclover, pea streak virus 1 and alfalfa mosaic viruses 1 and 1A produced a mild mottling. The infected plants became generally chlorotic and died. Alfalfa mosaic virus 1B produced local lesions on the inoculated leaves. The infected plants were decidedly stunted, became chlorotic, and died.

On crimson clover, pea streak virus 1 produced a marked mottling of the leaves (fig. 5, *O*), while alfalfa mosaic virus 1 produced a very mild mottling which frequently was difficult to diagnose. Alfalfa mosaic virus 1A produced an extreme mottling (*P*), the leaves being smaller than normal (*K*) and the plant stunted. Alfalfa mosaic virus 1B produced severe mottling, spotting, crinkling, and malformation of the leaves and a stunting of the plant (*M*).

On red clover, pea streak virus 1 produced a fairly distinct mottling. Alfalfa mosaic virus 1 manifested itself as a very mild mottling, while alfalfa mosaic virus 1A produced a mottling equal in intensity to that produced by pea streak virus 1. Alfalfa mosaic virus 1B likewise produced a distinct mottling and in addition a leaf spotting (fig. 5, *G*) similar to that on crimson clover. In this respect alfalfa mosaic virus 1B is distinctly different from any of the other viruses.

White lupine was resistant to pea streak virus 1, which can thus be distinguished from the other viruses. On white lupine the alfalfa mosaic viruses produced local lesions (fig. 5, *J* and *L*) that differed in size and number. Alfalfa mosaic virus 1 produced the smallest lesions and the fewest per unit area of leaf surface; alfalfa mosaic virus 1A produced the largest lesions and the greatest number per unit area. The inoculated leaves were killed most readily when inoculated with alfalfa mosaic virus 1A. The systemic symptoms produced by alfalfa mosaic virus 1 were a mild leaf mottling and a slight chlorosis. Alfalfa mosaic virus 1A produced a distinct mottling and considerable chlorosis. The uppermost leaves were small, puckered, distorted, and curled upward. Infected plants were decidedly stunted, and the growing tip was frequently killed. Alfalfa mosaic virus 1B produced symptoms slightly more pronounced than those produced by alfalfa mosaic virus 1.

Soybean was resistant to pea streak virus 1. Alfalfa mosaic virus 1 produced a stunting of the plant and a decided mottling, puckering, crinkling, arching, and downward curling of the leaflets; alfalfa mosaic virus 1A produced similar but slightly more intense symptoms (fig. 5, *D*). The youngest trifoliate leaves showed necrosis and frequently were killed; the plants, however, did not die. Alfalfa mosaic virus 1B produced symptoms more severe than those produced by alfalfa mosaic virus 1 but not so intense as those produced by alfalfa mosaic virus 1A.

The only virus that infected sweet pea was alfalfa mosaic virus 1A. The only symptom was a leaf mottling.

Alfalfa mosaic viruses 1A and 1B infected zinnia, producing a definite mottling of the leaves.

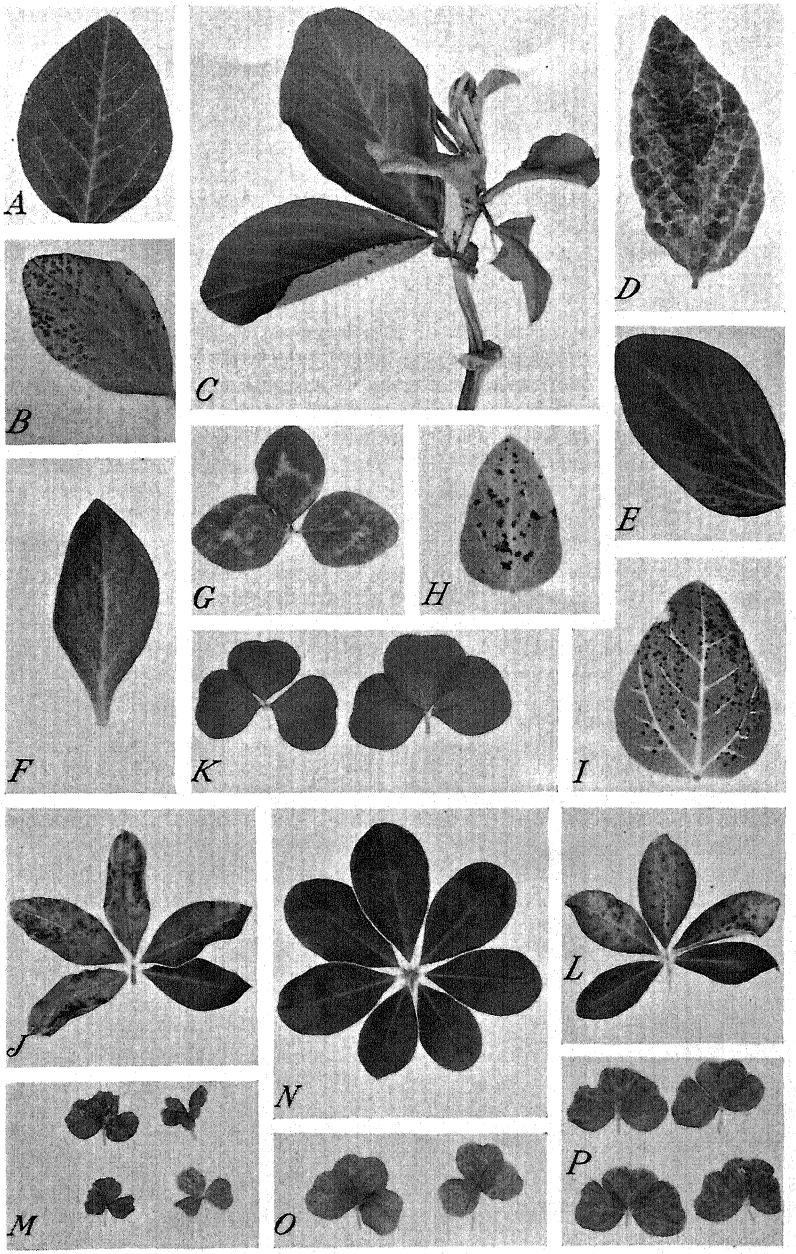


FIGURE 5.—Symptoms produced by pea streak virus 1 and alfalfa mosaic viruses on different hosts: *A*, Healthy soybean leaflet; *B*, *C*, variations in symptoms produced by alfalfa mosaic virus 1B on Windsor broadbean; *D*, mottling on soybean infected with alfalfa mosaic virus 1A; *E*, leaf spot on Windsor broadbean infected with alfalfa mosaic virus 1; *F*, mottling on petunia leaf caused by alfalfa mosaic virus 1A; *G*, red clover leaves infected with alfalfa mosaic virus 1B; *H*, *I*, local lesions produced by alfalfa mosaic viruses 1A and 1B, respectively, on Black Eye cowpea; *J*, *L*, white lupine infected with alfalfa mosaic viruses 1A and 1B, respectively; *K*, healthy crimson clover; *M*, *O*, *P*, crimson clover infected with alfalfa mosaic virus 1B, pea streak virus 1, and alfalfa mosaic virus 1A, respectively; *N*, healthy white lupine.

Tobacco was not infected by pea streak virus 1; the three alfalfa mosaic viruses produced similar symptoms, namely, white necrotic flecks and small rings and arcs on the inoculated leaves. A mottling later developed and was followed by necrotic areas similar to the oak-leaf pattern (fig. 3, *C*) of the tobacco ring spot virus described by Fromme et al. (*1*). The only symptomatological difference between the three viruses was in the intensity of the mottling. Alfalfa mosaic virus 1 produced the mildest mottling, and alfalfa mosaic virus 1A the most pronounced.

Jimsonweed was resistant to the pea streak virus but susceptible to the three alfalfa mosaic viruses. Alfalfa mosaic virus 1 produced a mild mottling, alfalfa mosaic virus 1A an intense mottling, and alfalfa mosaic virus 1B a still more intense mottling (fig. 3, *B*). The last-named virus produced also brown necrotic spots, which first appeared at the tip and later spread throughout the entire leaflet.

Petunia was not infected by the pea streak virus, but was susceptible to the three strains of alfalfa mosaic, each producing a decided mottling of the leaves. Alfalfa mosaic virus 1 caused the least intense mottling, while alfalfa mosaic viruses 1A and 1B produced severe mottling of about equal intensity (fig. 5, *F*). The leaves of the plants infected with alfalfa mosaic virus 1B were small, somewhat distorted, and necrotic.

Cucumber was infected only by alfalfa mosaic viruses 1A and 1B. The symptoms appeared on the young leaves as small chlorotic spots that gradually enlarged, causing a mild leaf mottling. On the later formed leaves, yellowish-green spots appeared throughout the leaf (fig. 3, *A*). Such leaves frequently showed extreme distortion.

PROPERTIES OF THE VIRUSES

Although the properties of alfalfa mosaic virus 1 had been previously studied by Pierce (*8*) and Zaumeyer and Wade (*20*), they were again determined in order to get a direct comparison of the properties of this virus with those of the pea streak virus and alfalfa mosaic viruses 1A and 1B. The thermal inactivation points, resistance to aging in vitro, and tolerance to dilution of the four viruses were studied for the purpose of determining differences as a means of separating and identifying them. In the case of the pea streak virus, the studies were made with peas as the test host. The determinations with alfalfa mosaic viruses 1 and 1A were made with beans because of the ability of the viruses to produce local necrotic lesions on them. In the trials with alfalfa mosaic virus 1B, both beans and peas were used. The routine methods described by Johnson and Grant (*4*) were employed; the only variation was in the aging tests. The viruses were stored in stoppered flasks and placed in a darkened constant-temperature chamber maintained at 20° C., whereas Johnson and Grant stored their viruses at temperatures ranging from 80° to 90° F.

Thermal Inactivation Point

The thermal inactivation point of pea streak virus 1 was found to be between 62° and 65° C. when heated for 10 minutes (table 4). Alfalfa mosaic virus 1 was inactivated between 65° and 70°. This is slightly higher than the inactivation point reported for the same virus by Pierce (*8*) and Zaumeyer and Wade (*20*), who found it to be

between 62° and 65°. Alfalfa mosaic virus 1A lost its infectivity at the same points as alfalfa virus 1. Alfalfa virus 1B was inactivated at 70° to 75°.

TABLE 4.—*Comparison of the thermal inactivation points of pea streak virus 1 alfalfa mosaic virus 1, alfalfa mosaic virus 1A, and alfalfa mosaic virus 1B*

Temperature (° C.)	Pea streak virus 1 on pea, systemic infection		Alfalfa mosaic virus				
			1 on bean, total lesions [1] produced by local infection	1A on bean, total lesions [1] produced by local infection	1B on—		
					Bean, total lesions [1] produced by local infection	Pea, systemic infection	
	Plants inoculated	Plants infected				Plants inoculated	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Not heated	20	20	5,860	5,880	4,560	12	12
58	25	7					
60	27	7	5,560	4,980		12	12
62	21	2	4,030	4,620	4,220	12	12
65	24	0	80	620	98	12	10
70	25	0	0	0	9	12	2
75						10	0

[1] Total number of lesions produced on 20 inoculated primary leaves of Stringless Green Refugee bean variety.

RESISTANCE TO AGING IN VITRO

Pea streak virus 1 was noninfectious when aged 2 days at 20° C. (table 5); alfalfa mosaic virus 1 was inactivated after aging 4 to 5 days. This is not in agreement with Pierce (*8*), who reported the virus to be infectious after 8 to 9 days' aging at laboratory temperatures. Zaumeyer and Wade (*20*) previously reported the virus as living 3 to 4 days in vitro. Alfalfa mosaic viruses 1A and 1B were noninfectious after 4 to 5 days' aging.

TOLERANCE TO DILUTION

Table 6 shows that pea streak virus 1 was infectious at a dilution of 1 to 5,000. Alfalfa mosaic virus 1, however, lost its activity at a dilution greater than 1 to 2,000, which agrees with the results of both Pierce (*8*) and Zaumeyer and Wade (*20*). Alfalfa mosaic viruses 1A and 1B were infectious at a dilution of 1 to 3,000 but not at 1 to 5,000.

The differences in the properties of the several viruses seem to be sufficient to differentiate pea streak virus 1 from the alfalfa mosaic viruses. The thermal inactivation point for the pea streak virus was lower than that of the three strains of the alfalfa mosaic virus. Regarding aging in vitro, pea streak virus 1 lost its infectivity more rapidly, but tolerated a higher dilution than did the alfalfa mosaic viruses.

Alfalfa mosaic viruses 1, 1A, and 1B showed variations in their physical properties; these variations, however, were too slight to be of significance in differentiating the viruses. The only variation worth noting was that alfalfa mosaic virus 1B was inactivated at a slightly higher temperature than alfalfa mosaic viruses 1 and 1A.

TABLE 5.—*Comparison of resistance to aging in vitro of pea streak virus 1, alfalfa mosaic virus 1, alfalfa mosaic virus 1A, and alfalfa mosaic virus 1B*

Time aged at 20° C.	Pea streak virus 1 on pea, systemic infection		Alfalfa mosaic virus				
					1B on—		
	Plants inoculated	Plants infected	1 on bean, total lesions [1] produced by local infection	1A on bean, total lesions [1] produced by local infection	Bean, total lesions [1] produced by local infection	Pea, systemic infection	
						Plants inoculated	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
None	12	12	9,260	4,025	3,654	12	12
6 hours	13	12					
8 hours	13	11					
10 hours	14	10					
12 hours	15	8					
24 hours	11	1	30	140		12	12
2 days	11	0	3	4	4	10	1
3 days			5	4	2	12	0
4 days			3	3	2	12	2
5 days			0	0	0	10	0
7 days							

[1] Total number of lesions produced on 20 inoculated primary leaves of Refugee Green bean variety.

TABLE 6.—*Comparison of tolerance to dilution of pea streak virus 1, alfalfa mosaic virus 1, alfalfa mosaic virus 1A, and alfalfa mosaic virus 1B*

Dilution	Pea streak virus 1 on pea, systemic infection		Alfalfa virus 1 on bean, total lesions [1] produced by local infection	Alfalfa virus 1A on bean, total lesions [1] produced by local infection	Alfalfa virus 1B on—		
					Bean, total lesions [1] produced by local infection	Pea, systemic infection	
	Plants inoculated	Plants infected				Plants inoculated	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
None	20	20	9,860	4,730	14,100	10	10
1:500	23	18	30	29	96	10	10
1:1,000	21	14	6	32	11	10	8
1:2,000	22	11	3	7	2	13	9
1:3,000	28	12	0	4	2	13	2
1:5,000	17	6		0	0	14	0

[1] Total number of lesions produced on 20 inoculated primary leaves of Refugee Green bean variety.

SEPARATION OF THE VIRUSES

The separation of the several viruses may be accomplished by means of physical-property studies as well as by differences in host range.

Pea streak virus 1 can be separated from a mixture with the alfalfa mosaic viruses by inoculation to the Horal variety of peas, which is susceptible to the pea streak virus but immune to the three strains of alfalfa mosaic virus. On the other hand, it is possible to isolate the alfalfa mosaic viruses from a mixture with pea streak virus 1 by inoculating garden bean, soybean, cowpea, white lupine, tobacco, petunia, and jimsonweed, which are all susceptible to the three strains of alfalfa mosaic virus, but resistant to pea streak virus 1.

Aging the viruses in vitro over 25 hours at 18° C. will separate pea streak virus 1 from the alfalfa mosaic viruses, since the latter are active for about 3 days under such conditions, while pea streak virus 1 is inactivated when aged for more than 25 hours at 18°.

Although the three alfalfa mosaic viruses are distinguishable from one another on many hosts, it is difficult to separate them in a mixture.

Thus far no method has been found of recovering alfalfa mosaic virus 1 from a mixture with alfalfa mosaic viruses 1A and 1B. Alfalfa mosaic viruses 1A and 1B can be obtained from a mixture with alfalfa mosaic virus 1 by inoculating zinnia or cucumber, which are susceptible to alfalfa mosaic viruses 1A and 1B but resistant to alfalfa mosaic virus 1, or by diluting a virus mixture 1 to 3,000, a dilution at which alfalfa mosaic virus 1 is inactivated while the other two viruses are not.

Alfalfa mosaic virus 1A can be separated from alfalfa mosaic virus 1 and 1B by inoculating sweet pea, Pink Cockade variety, which is susceptible to alfalfa mosaic virus 1A but resistant to the other two viruses.

Alfalfa mosaic virus 1B produces a necrotic spotting of the leaves on many pea varieties. It can be separated from a mixture of the alfalfa mosaic viruses by diluting an extract 1 to 1,000 and inoculating the Capucijner pea variety, on which it produces a necrotic spotting of the leaves above the point of inoculation. By removing this necrotic tissue, reinoculating the same variety with the expressed juice of the necrotic tissue, and repeating this procedure, alfalfa mosaic virus 1B can be separated from alfalfa mosaic viruses 1 and 1 A.

DISCUSSION

Within the past few years a number of investigations (*7*, *8*, *13*, *17*, *20*, *21*, *22*) have shown that several distinct viruses are capable of infecting peas, beans, and other legumes. Certain of these viruses produce similar symptoms on various hosts and are therefore difficult to differentiate. Host range and property studies, together with symptomatological comparisons, have made it possible to name, describe, and classify certain of the viruses affecting legumes.

The evidence presented in this paper shows that, in addition to those viruses already described on legumes, still others are infectious to them. The data also show that the four viruses described can be differentiated, even though the symptoms they produce on certain hosts are in some cases and under certain conditions quite similar. In table 7 most of the outstanding differences in host reaction are noted.

TABLE 7.—*Summarized table for the differentiation and identification of pea streak virus 1 and alfalfa mosaic viruses 1, 1A, and 1B*

Virus	Type of infection	Reaction [1] of—																
		Phaseolus vulgaris (Stringless Green Refugee variety)	*Pisum sativum* (Perfection variety)	*Pisum sativum* (Horal variety)	*Vicia faba* (Windsor variety)	*Vigna sinensis* (Groit variety)	*Melilotus alba*	*Trifolium incarnatum*	*Trifolium pratense*	*Lupinus albus*	*Soja max*	*Lathyrus odoratus*	*Cucumis sativus*	*Datura stramonium*	*Nicotiana tabacum*	*Petunia hybrida*	*Zinnia elegans*
Pea streak virus 1	Local	—	—	—	b	—	—	—	—	—	—	—	—	—	—	—	—
	Systemic	—	a	b	a	—	c	a	b	—	—	—	—	—	—	—	—
Alfalfa mosaic virus 1.	Local	a	—	—	c	c	c	c	c	b	b	—	—	c	c	c	—
	Systemic	—	c	—	b	—	—	—	—	a	—	—	—	—	—	—	—
Alfalfa mosaic virus 1A.	Local	a	—	—	b	b	—	a	—	a	—	—	—	b	c	c	—
	Systemic	—	b	—	a	—	c	a	b	a	a	c	b	b	a	b	b
Alfalfa mosaic virus 1B.	Local	a	—	—	a	b	c	—	—	b	—	—	b	a	b	a	—
	Systemic	—	a	—	a	—	c	a	a	b	b	—	b	a	b	a	b

[1] Minus sign (—) represents no infection; a, severe infection; b, moderate infection; c, mild infection.

It is quite likely that some of the legume viruses thus far described, as well as others that may be described later, are not necessarily new viruses but are actually strains of some that have already been described, even though they may have been isolated from different hosts. For example, the three alfalfa mosaic viruses described here, though closely related, possess certain characteristics that distinguish them from one another. It is not unlikely that others will be found differing from those herein described.

Since 1928, a number of viruses causing pea streak have been reported. In comparing the symptoms produced by several of these, one might be led to believe that they are the same. Thus the symptoms of pea streak virus 1 described in this paper show marked similarity to those of the pea streak described by Linford [6] in the United States in 1928, and later from Hawaii. Linford (*5*) proved that the pea streak virus in Hawaii was identical with the yellow spot virus of pineapple and suggested that the pea streak in the United States may be caused by either this or a related virus. What appeared to be the same disease was observed by the writer in pea fields in Idaho, in 1932 and 1934. In 1934 it was also reported [7] from Montana, where a field of peas showed a considerable amount of infection, so much, in fact, that the yield was materially reduced. L. K. Jones, in a bulletin by Vincent (*14*), reported a pea streak virus in western Washington in 1935.

The present writer observed that infected pea plants were most commonly found in close proximity to alfalfa plants, either where volunteer alfalfa plants appeared in a pea field or where alfalfa was growing along the borders of a field. It was also reported [7] that the disease in Montana was found in a pea field planted to alfalfa in previous years and that a considerable number of volunteer alfalfa plants occurred there. No virus determination was possible at the time, so it is not known whether this disease was similar to the streak disease reported in this paper or to the disease which Linford (*5*) described on peas from Hawaii. Since alfalfa plants have been associated in most cases with the disease in the United States, it appears that these plants may have some logical connection with the disease. The alfalfa plants showed no signs of being infected with a virus, but the symptoms may have been masked, as is frequent under conditions of high temperature.

If the pea streak disease in the western part of the United States is the same as that reported by Linford (*5*, *6*) in Hawaii, it is distinctly different from pea streak virus 1 herein described. The pea streak virus from Hawaii is transmitted by *Thrips tabaci* and is not readily transmitted artificially. Pea streak virus 1 is transmitted by the pea aphid (*Illinoia pisi*) and in preliminary tests was not shown to be transmitted by *T. tabaci*. The virus is readily transmitted by artificial means.

The spotted wilt of garden pea reported by Whipple (*17*) appears to be the same disease as Linford's (*6*) pea streak and pineapple yellow spot. The symptoms of the spotted wilt virus on pea resemble those produced by pea streak virus 1, and the symptoms caused by the former virus on *Vicia faba*, as reported by Smith (*11*), resemble those of pea streak virus 1 on this host. However, differences in host range, properties, and transmission indicate that the viruses are not related.

[6] Linford, Maurice B. See footnote 3, p. 747.
[7] Correspondence in files of Division of Fruit and Vegetable Crops and Diseases.

Snyder's (*12*) spotted wilt of sweet pea is identical with Whipple's (*17*) virus of garden pea.

Pierce (*7*) isolated a virus from a red clover plant, which he named the broadbean local-lesion virus. When inoculated to broadbean, it produced local necrotic lesions but no systemic symptoms. He stated that the local-lesion virus, especially when in combination with other viruses, tends to produce streak symptoms on peas.

Although Pierce's (*7*) host-range studies with this virus were meager, there appear to be minor similarities between pea streak virus 1 and the broadbean local-lesion virus. Both viruses produce local necrotic lesions on *Vicia faba*, but pea streak virus 1 in addition becomes systemic. Both infected soybean but were not infectious to Stringless Green Refugee bean.

Regarding the properties of the viruses, the broadbean local-lesion virus is inactivated when heated for 10 minutes at 60° to 62° C., and on aging in vitro from 2 to 3 days. Pea streak virus 1 is inactivated on heating to 62° to 65° and on aging from 1 to 2 days.

It is probable that these viruses are similar and that the local-lesion virus of Pierce may be a less virulent strain of pea streak virus 1. A more comprehensive study of the host range and properties of the broadbean local-lesion virus is necessary before this can be definitely determined. The alfalfa mosaic viruses discussed in this paper differ distinctly from the broadbean local-lesion virus.

Alfalfa mosaic virus 1, reported in this paper, is identical with the alfalfa virus described by Weimer (*15*, *16*), as well as with the alfalfa virus reported by Zaumeyer and Wade (*20*). It is likewise the same virus as that described by Pierce (*8*) as alfalfa virus 2.

The viruses of white clover mosaic and sweetclover mosaic, producing streak symptoms on peas, reported by Zaumeyer and Wade (*19*, *22*) and Pierce (*7*), are dissimilar to the viruses described herein. Stubbs (*13*), who produced a streak symptom on peas with the tobacco ring spot virus, dealt with a virus unlike those described in this paper.

The alfalfa mosaic viruses discussed here are of peculiar interest because of their wide host range and because of the fact that the symptoms they produce on certain hosts are somewhat similar to those produced by other apparently unrelated viruses. As pointed out earlier, the alfalfa mosaic viruses infect certain hosts, outside of the family Leguminosae, which have not been shown to be infected by the previously described legume viruses.

The local lesions produced by the tobacco mosaic virus on beans, as shown by Price (*9*), are somewhat similar to though smaller than those produced by the alfalfa mosaic viruses. Similarly, the local necrotic lesions produced on cowpea by the various strains of cucumber mosaic, as pointed out by Price (*10*), are in general quite similar to the lesions of the alfalfa mosaic viruses on this host (fig. 5, *H* and *I*).

The alfalfa mosaic viruses produce on the inoculated leaves of Turkish tobacco small white rings and arcs, not unlike those produced by the course etch virus of Johnson (*3*) on burley tobacco. On the uppermost leaves they produce white concentric rings and patterns, resembling those produced by the ring spot virus from sweetclover described by Henderson (*2*).

On petunia the alfalfa mosaic viruses produce symptoms that resemble the early symptoms of the cucumber mosaic virus on this host.

They are, however, much less pronounced than the symptoms produced by the tobacco mosaic virus and the tobacco ring spot virus.

On *Datura stramonium* the symptoms of the alfalfa mosaic viruses slightly resemble those produced by the latent or X virus of potato.

The early mottling produced by the alfalfa mosaic viruses on cucumber is slightly similar to the mottling exhibited by cucumber mosaic. Later, however, the symptoms of the alfalfa mosaic viruses are more severe and are distinctly different from those produced by the cucumber virus. Infected plants are stunted and the leaves become dark green with small stipplelike chlorotic spots. Later formed leaves are decidedly puckered and savoyed and much smaller than normal. The yellow areas are not so well defined in leaves infected with cucumber mosaic virus as in those infected with the alfalfa mosaic viruses.

Insect transmission of the alfalfa mosaic viruses was not studied. It was shown that pea streak virus 1 was transmitted by the pea aphid, a feature which distinguished it from the spotted wilt virus. Weimer (*16*) showed that alfalfa virus 1 was transmitted from diseased to healthy alfalfa plants by the pea aphid. It is not unlikely that this aphid can transmit this virus, as well as alfalfa mosaic viruses 1A and 1B, to pea.

The economic importance of pea streak virus 1 is unknown. If the pea streak disease found by the writer and others in commercial fields is similar to pea streak virus 1 or to any of the alfalfa mosaic viruses, then it is of commercial importance.

Alfalfa mosaic is widespread and has been reported from many localities. The symptoms are quite noticeable in the spring and fall of the year throughout the eastern and midwestern United States where the disease has been found; but in summer, when the temperatures are high, it is difficult to diagnose. Infected plants appear normal under these conditions, but the virus is readily isolated from such plants. L. K. Jones (as reported by Vincent (*14*)) found alfalfa mosaic abundant in certain counties of Washington, and he believes that the disease causes a decline in vigor and possibly is a factor in winter injury of the plants.

The relationship of alfalfa mosaic to peas under field conditions is unknown. Because of the similarity of symptoms of the alfalfa mosaic viruses and certain of the other legume viruses on peas, it is not unlikely that the alfalfa mosaic may be responsible, in some localities, for at least part of the reported pea mosaic. Alfalfa mosaic has never been found to affect beans under field conditions and hence is no factor in crop production.

From the foregoing data, it is evident that the viruses herein described are different from those heretofore reported as infecting pea and that they can readily be distinguished from one another as well as from other viruses affecting pea.

SUMMARY

A new virus disease of pea, of the streak type, together with a comparison of three strains of alfalfa mosaic that are infectious to pea, bean, and other legumes, are described and identified.

The differentiation of the viruses was based on the expression of symptoms produced on numerous hosts, varietal resistance of pea and bean, host range, and properties of the viruses in vitro.

Pea streak virus 1 produced a streaking of the stems, petioles, and main veins of the leaves of the pea, but no leaf mottling. All of the alfalfa mosaic viruses produced a leaf mottling, and in addition alfalfa mosaic virus 1B caused a spotting of the leaves and a slight streaking of the stems.

Seventeen varieties of peas were tested with the four viruses. All varieties were susceptible to pea streak virus 1. Horal was the only variety immune to the three alfalfa mosaic viruses. In general, alfalfa mosaic virus 1B produced the severest symptoms of the alfalfa mosaic viruses.

Pea streak virus 1 was not infectious to bean, whereas the alfalfa mosaic viruses produced local necrotic lesions. The Stringless Green Refugee variety was the most susceptible to the three alfalfa mosaic viruses, as determined by total number of lesions produced. Great Northern U. I. No. 1 was next in susceptibility. Corbett and Robust showed about the same tolerance.

The host range of the pea streak virus is more limited than that of the three strains of alfalfa mosaic virus. Pea streak virus 1 did not infect any species outside of the family Leguminosae. The alfalfa mosaic viruses, besides infecting many legumes, also infected *Nicotiana tabacum, Petunia hybrida, Datura stramonium, Cucumis sativus,* and *Zinnia elegans.*

The symptoms produced by the four viruses on certain differential hosts showed distinct differences, making it possible further to distinguish the viruses.

The thermal inactivation point, resistance to aging in vitro, and tolerance to dilution were determined for all of the viruses. The pea streak virus was inactivated between 62° and 65° C. Alfalfa mosaic viruses 1 and 1A were not infectious when heated between 65° and 70° for 10 minutes. Alfalfa mosaic virus 1B was inactivated when heated at 70° to 75° for 10 minutes. Pea streak virus 1 lost its infectivity when aged in vitro for 2 days. The alfalfa mosaic viruses were noninfectious after 4 to 5 days' aging. Pea streak virus 1 tolerated a dilution of about 1 to 5,000. Alfalfa mosaic virus 1 lost its activity at a dilution of about 1 to 2,000 and alfalfa mosaic viruses 1A and 1B at about 1 to 3,000.

Pea streak virus 1 can be separated from a mixture with the alfalfa mosaic viruses by inoculating Horal peas. This variety is susceptible to the pea streak virus but immune to the alfalfa mosaic viruses. No means of separating alfalfa mosaic virus 1 from a mixture with alfalfa mosaic viruses 1A and 1B has been found. The separation of alfalfa mosaic viruses 1A and 1B from each other, as well as from alfalfa mosaic virus 1, can be accomplished by differential host reactions as well as by differences in their properties in vitro.

The differences between the several viruses here reported, as well as between other legume viruses, appear to be sufficient to permit identification and classification.

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STUDIES ON THE CAUSE OF IMMUNITY OF MONOCOTYLEDONOUS PLANTS TO PHYMATOTRICHUM ROOT ROT [1]

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INTRODUCTION

The root rot disease caused by *Phymatotrichum omnivorum* (Shear) Duggar is known to attack 1,708 kinds of plants (*8*).[3] All of these are gymnosperms or dicotyledons. Though some dicotyledonous plants are resistant to root rot, the majority are moderately to extremely susceptible. No monocotyledonous plant, however, has yet been proved to be susceptible to this disease (*7*).

Earlier results (*3*) indicate that this general immunity of monocotyledons to *Phymatotrichum* root rot is associated with a relatively high concentration in their roots of some substance that inhibits the growth of *P. omnivorum*. Little, if any, growth of the fungus occurred in autoclaved, undiluted juices from the roots of four immune monocotyledons, namely, corn (*Zea mays* L.), onion (*Allium cepa* L.), canna (*Canna* sp.), and nutgrass (*Cyperus rotundus* L.). On the other hand, profuse growth was obtained in autoclaved, undiluted juices from cotton (*Gossypium hirsutum* L.), carrot (*Daucus carota* L.), and sweetpotato (*Ipomoea batatas* (L.) Lam.) roots. After the juices had been diluted with distilled water, good growth was obtained in material from both the immune and susceptible plants.

These results have since been confirmed by further tests (*2*) by the same methods. With the dicots, there was again heavy growth in undiluted juices from cotton, sesbania (*Sesbania* sp.), guar (*Cyamopsis psoraloides* (Lam.) DC.), and carrot roots, with less growth as the juices were diluted with distilled water. With the monocots, undiluted juices from onion, nutgrass, and sorghum (*Sorghum vulgare* Pers.) roots nearly or entirely prohibited growth, while diluted juices permitted good growth. However, somewhat aberrant results were obtained with juices from cowpea (*Vigna sinensis* (L.) Endl.) and corn roots. The general nature of the results is shown in part in figure 1. Colonies of *Phymatotrichum omnivorum* here reached the following mean weights in 28 days: In undiluted carrot juice, 593 mg; in carrot juice diluted to one-fourth in distilled water, 242 mg; in undiluted onion juice, no growth; and in diluted onion juice, 181 mg.

Since dilution of juice from monocots permitted growth, the inhibition in the undiluted juice could scarcely have been due to insufficient nutrients. It appears probable instead that specifically

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[3] Reference is made by number (italic) to Literature Cited, p. 786.

inhibitory materials present in these juices make monocots immune to *Phymatotrichum* root rot.

Without discussing work on the nature of resistance to various plant diseases, recently reviewed at length by Brown (*1*), mention should be made of studies by Moore (*6*) on the rate of growth of *Phymatotrichum omnivorum* in dilute decoctions prepared from dried roots. Decoctions from cotton roots, added to potato-dextrose agar, accelerated growth, while fresh decoctions from corn, wheat, or

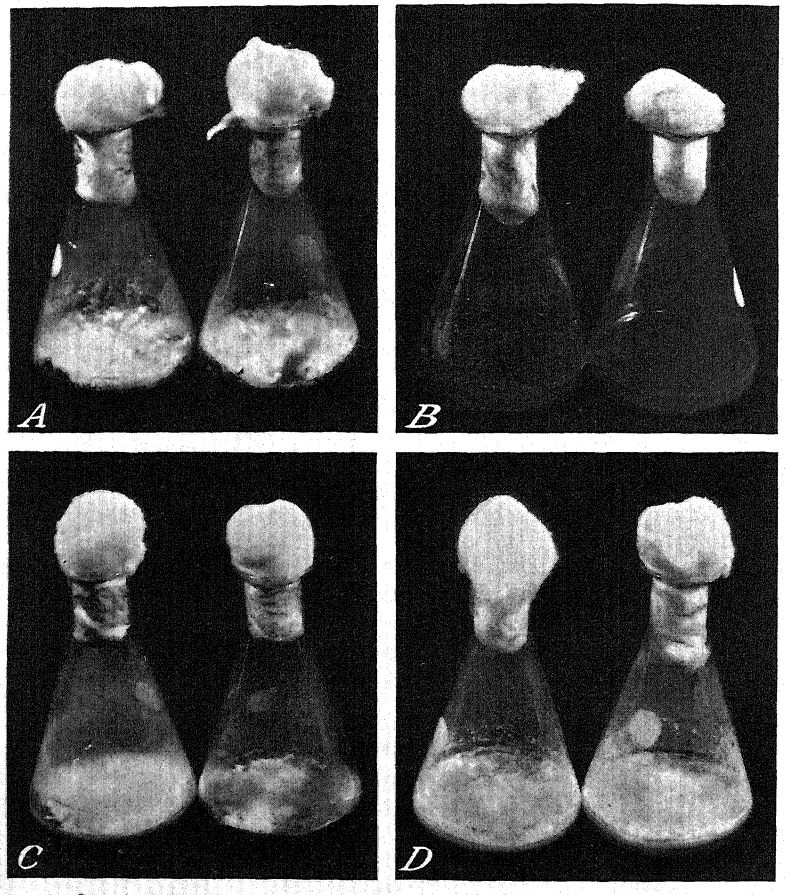


FIGURE 1.—Growth of *Phymatotrichum omnivorum* after 4 weeks in juices expressed from carrots (*A*, *C*) and onions (*B*, *D*). *A*, *B*, undiluted juices; *C*, *D*, diluted to one-fourth in distilled water. Note no growth in undiluted onion juice (*B*) and good growth after dilution (*D*).

barley roots generally retarded it. Moore concluded that "water-soluble, labile substances present in the roots of plants immune from the attack of Phymatotrichum have some part in establishing this immunity."

The present paper summarizes studies directed toward isolation and identification of the inhibitory substances in roots of monocotyledonous plants. Certain aspects of this work have been presented previously in abstract form (*5*).

METHODS

The general method of attack has been to attempt to grow the fungus, *Phymatotrichum omnivorum*, on juices expressed from the roots of various plants, and on combinations of juices and fractions of juices with a synthetic nutrient solution. The fungus was grown in small volumes of these liquids in culture tubes. Fractions of plant juices were compared on the basis of the concentrations necessary to inhibit growth. Growth was always successful in check tubes containing the synthetic nutrient solution alone.

In the preparation of the juices, only the underground portions of plants (roots, corms, rhizomes, etc.) were used. These were washed, ground, and the juice expressed with a hand press. The juice was filtered through absorbent cotton and used at once for fractionation as outlined below in presentation of the various experiments. The solvents used in fractionation prevented fungous or bacterial contamination during manipulation, but no protection against changes due to oxidation was attempted.

Final fractions of juices were added to synthetic nutrient solution 70 (*4*), which contains per liter (grams): Ammonium nitrate, 1.18; dextrose, 40; K_2HPO_4, 1.35; $MgSO_4.7H_2O$, 0.75; KCl, 0.15; and $FeCl_3$, 0.0015. The nutrient solution was used in uniform concentration in all the final cultures, while the concentration of the plant juice fraction was varied throughout the range indicated in the tables. In general, the final media were adjusted uniformly to pH 7.0 by the addition of potassium hydroxide when required. Ether-soluble materials, added in ether solution, were usually not neutralized, but were added in relatively minute amounts to the buffered nutrient solution. The media were distributed to culture tubes, and autoclaved at 15 pounds for 20 minutes. Except as otherwise specified, this final autoclaving was the first heating of the material.

Ether-soluble fractions of juices have generally been dispensed while still in ether solution. Individual portions were placed in culture tubes, which already contained 1 cc of the synthetic nutrient solution, and the tubes were warmed at reduced pressure to remove the ether. The remaining 3- or 4-cc portion of nutrient solution was then added to each tube, and the tubes were plugged and autoclaved.

Aqueous fractions of the plant juices were concentrated at reduced pressure, mixed as required with nutrient solution to obtain various concentrations of the plant-juice material, and the mixture was then measured into the culture tubes.

The cultures were inoculated (except in a few experiments) with portions of sclerotial masses of *Phymatotrichum omnivorum* strain 24 taken from flask cultures on synthetic media, and were incubated at room temperature. Observations on possible growth were continued for several months, the tables representing the maximum growth possible rather than growth within a definite period. Lack of growth of the fungus, shown by minus signs in the tables, indicated that a particular dilution of the plant juice was capable of completely inhibiting growth of the fungus. Growth indicated lack of potency. In the tables, a single plus sign represents growth that filled not more than half of the culture solution; two plus signs represent growth that filled most of the culture solution. Cultures were replicated four, five, or occasionally six times, and there was generally little variation in

results within a group of replicates. Some variation was to be expected near the concentration limiting growth of the fungus, particularly with fractions not soluble in water but of necessity tested in the aqueous nutrient solution.

In tabulating the results, the concentrations mentioned for various fractions of the plant juices in the culture solutions refer always to the volume of original plant juice from which the fraction was prepared, without regard to the weight of material in this particular fraction. A fraction containing only a trace of the original dry material was still considered of 1.0 concentration if made up to the volume of the juice prior to fractionation. Similarly, a concentration of 12.0 in the tables means that the final substratum contained a fraction prepared from a volume of plant juice equal to 12 times the volume of the culture solution, even though the culture solution may have contained only 1 percent of the dry matter present in this original juice. The dry weights, given for some of the fractions, refer to grams per 100 cc of the fraction at 1.0 concentration.

EXPERIMENTAL RESULTS

ETHER-SOLUBLE AND AQUEOUS FRACTIONS OF PLANT JUICES

Several preliminary fractionations of various juices may be summarized briefly. Fresh onion juice was treated with phosphotungstic acid, filtered, the filtrate cleared, treated with neutral lead acetate, and the precipitate again removed. Neither precipitate inhibited growth of *Phymatotrichum omnivorum*. The filtrate was deleaded, evaporated to a thick paste, and extracted with absolute alcohol. The extract proved nonpotent in preventing growth of the fungus, while the residue was highly potent. Acetone and ether extracts from the residue were potent. Ether extracts from an aqueous solution of the residue were inhibitory, but even after exhaustive ether extraction which yielded finally nonpotent extracts, the aqueous residue was still potent.

Fresh onion juice was extracted repeatedly with ether, with similar results. A first extract by two portions of ether was highly potent, preventing growth of *Phymatotrichum omnivorum* at a concentration of 1.5. The aqueous residue was acidified with hydrochloric acid and extracted with two more portions of ether, yielding a somewhat less potent fraction, able to prevent growth at 3.0. The aqueous residue was then made alkaline with potassium hydroxide and extracted again, yielding a nonpotent ether fraction. The final aqueous residue was still potent, preventing growth at a concentration of 3.0. These results indicated that onion juice contained both ether-soluble and nonether-soluble materials able to inhibit growth of *Phymatotrichum omnivorum*.

Fresh juice from corn roots was extracted similarly with ether. As with onion, the ether extract (from acidified juice) and the aqueous residue were about equally potent in preventing growth of the fungus.

Some experiments were performed to compare fractions prepared in this way from monocots and dicots. In the series summarized in table 1, the aqueous residue of the juices did not differentiate between susceptible and immune plants. This fraction prevented growth in material from the highly susceptible carrots and from the immune onions. The ether extract, on the other hand, was potent with the onion but did not inhibit growth with juices from either of the dicots.

TABLE 1.—*Inhibitory effect of some fractions of carrot, turnip, and onion juice on growth of Phymatotrichum omnivorum*

Juice and fraction used	Growth (+) or lack of growth (−) in nutrient solution 70 plus the following concentrations[1] of fractions—				
	6.0	3.0	1.5	1.0	0.75
Carrot:					
Complete juice, adjusted to pH 7.0	----------	----------	----------	++	++
Ether extract	++	++	++	----------	++
Aqueous residue	−	−	++	----------	++
Turnip:					
Complete juice, adjusted to pH 7.0	----------	----------	----------	−	−
Ether extract	++	++	++	----------	++
Aqueous residue	−	−	−	----------	−
Onion:					
Complete juice, adjusted to pH 7.0	----------	----------	----------	++	++
Ether extract	−	−	−[2]	----------	−[2]
Aqueous residue	−	−	++	----------	++

[1] Concentrations represent the volume of the original juice from which a portion of the fraction was prepared, divided by the volume of the culture solution in which that portion is tested.
[2] Growth in 1 of 5 tubes.

In other experiments carried out at this time, ether extracts were prepared from juices from cannas, onions, potatoes, sweetpotatoes, carrots, and *Hemerocallis* roots. In sufficiently high concentration, the aqueous residues remaining after ether extraction of all these juices inhibited growth of *Phymatotrichum omnivorum*. On the other hand, the ether-soluble fractions from the juices of the monocots were generally potent, while those from the juices of the susceptible dicots were rarely inhibitory, even when used at high concentration. This suggested that the materials that make monocots immune from root rot might be those present in the ether-soluble fractions of the juices, rather than those in the aqueous fractions which were apparently common to both groups of plants. Further attention was therefore given to the ether fractions.

ETHYL ETHER AND PETROLEUM ETHER FOR SEPARATION OF POTENT FRACTIONS

In experiments with onion juice, canna root juice, and *Hemerocallis* root juice, extraction with ethyl ether was compared with extraction with petroleum ether. In each case ethyl ether extracts were more potent in preventing growth of *Phymatotrichum omnivorum* than extracts with petroleum ether. When the same portion of juice was extracted first with petroleum ether and then with ethyl ether, the ethyl ether fraction was much more potent than the petroleum ether fraction. On the other hand, when juice was extracted first with ethyl ether and then with petroleum ether, all the potent material was extracted by the ethyl ether (table 3). Extraction with ethyl ether was thus more complete and included the materials that might be taken out by petroleum ether. Ethyl ether was therefore used for later extractions.

ACETONE PRECIPITATION OF ETHER-SOLUBLE FRACTIONS

Phospholipins were separated by treating ether extracts with anhydrous acetone in large volume overnight in the refrigerator. The white, greasy phospholipin precipitate obtained from ether extracts

from onion, corn, *Hemerocallis*, and other monocots invariably proved nonpotent (fraction *c*, table 2), while the acetone-soluble materials remaining in the ether-acetone solution (fraction *d*) were as potent in preventing growth of *Phymatotrichum omnivorum* as the original complete ether extract.

TABLE 2.—*Inhibitory effect of some fractions of onion juice on growth of Phymatotrichum omnivorum*

Treatment of the fractions	Percent dry weight [1]	Growth (+) or lack of growth (−) in nutrient solution 70 plus the following concentrations of fractions—						
		12.0	9.0	6.0	3.0	1.5	1.0	0.75
a. Complete juice, at pH 7.0	6.9475	------	------	------	------	------	−	−
b. Complete ether extract	.0324	−	−	−	−	−	------	------
Precipitation of *b* with acetone:								
c. Precipitate	.0056	+	+	++	++	++	------	------
d. Acetone-soluble	.0268	−	−	−	−	−	------	------
Saponification of *d* for 2 hours at room temperature:								
e. Nonsaponified	.0223	−	−	−	++	++	------	------
f. Saponified, ether-soluble	.0056	−	−	−	++	++	------	------
g. Saponified, aqueous residue	--------	++	++	++	++	++	------	------

[1] Dry weights refer to grams per 100 cc of the fraction at 1.0 concentration.

SAPONIFICATION WITH ALCOHOLIC POTASSIUM HYDROXIDE

Saponification of ether extracts with alcoholic potassium hydroxide was tried in several experiments. After saponification, water was added and an ether extraction was made which presumably removed nonsaponified materials. The aqueous residue was then acidified with hydrochloric acid, and a second ether extraction was made to remove acidic, saponified materials. The final aqueous residue consisted of saponified materials, soluble in water but not in ether.

Fractionation of onion juice in this way usually yielded results as given in table 2 for one experiment. The nonsaponified fraction (*e*) was reduced in weight and potency by longer treatment, or higher temperatures during saponification, but usually retained some potency. Most of the potency of the saponified material was in the ether-soluble fraction (*f*).

Similar fractionation of the ether-acetone-soluble portion of corn juice yielded different results. The nonsaponified portion lost all potency, but the saponified, ether-soluble fraction was as potent as the material prior to saponification. This apparent discrepancy in saponification of ether fractions from onion and those from other monocot juices was explained in later fractionations in aqueous sodium carbonate solution.

These results indicated that the potency of ether extracts of monocots was due at least in part to saponifiable materials containing an acidic constituent.

FRACTIONATION OF ETHER EXTRACTS BY EXTRACTION WITH AQUEOUS SODIUM CARBONATE SOLUTION

The potency of ether extracts from plant juices was not diminished by repeated washing with distilled water. Washing with relatively small volumes of 1-percent aqueous sodium carbonate solution, how-

ever, caused extreme reduction in potency. For example, an ether extract from canna root juice contained 0.0081 g per 100 cc of the original material, and prevented growth of *Phymatotrichum omnivorum* when present at a concentration of 6.0. After washing twice with sodium carbonate solution and again with distilled water, the dry matter had decreased only to 0.0050 g per 100 cc of the original juice, while no potency was found even at a concentration of 12.0.

TABLE 3.—*Inhibitory effect of some fractions of juice from Hemerocallis roots on growth of Phymatotrichum omnivorum*

Treatment of the fractions	Percent dry weight [1]	Growth (+) or lack of growth (−) in nutrient solution 70 plus the following concentrations of fractions—							
		12.0	9.0	6.0	3.0	1.5	1.0	0.75	0.25
a. Complete juice, at pH 7.0	-------	------	------	------	------	------	−	−	++
Extraction with petroleum ether, followed by ethyl ether:									
b. Petroleum ether extract	0.0023	−	[2]+	++	++	++	------	------	------
c. Ethyl ether extract	.0308	−	−	−	++	++	------	------	------
Extraction with ethyl ether followed by petroleum ether:									
d. Ethyl ether extract	.0257	−	−	−	++	++	------	------	------
e. Petroleum ether extract	.0019	++	++	++	++	++	------	------	------
f. Aqueous residue from *d*	-------	−	−	−	−	−	------	------	------
Washing *d* with 1 percent sodium carbonate solution:									
g. Residue from *d* after washing	.0115	++	++	++	++	++	------	------	------
h. Ether extract from acidified washings	.0120	−	−	−	[2]++	++	------	------	------

[1] See footnote 1, table 2.
[2] Growth in 2 of 4 tubes only.

Results typical of those obtained with juices from various monocots are given in table 3 for *Hemerocallis* roots. In this experiment, the complete ethyl ether extract (*d*) was potent enough to prevent growth when at 6.0 concentration. Washing 900 cc of this fraction with two 250-cc portions of 1-percent sodium carbonate solution, and with one 250-cc portion of distilled water, reduced the weight of dry matter in the extract (*g*) by a half and entirely removed the potency. The combined washings were acidified slightly with hydrochloric acid, and extracted with three 100-cc portions of ether. The inhibitory material was recovered in a fraction (*h*) containing less than half the dry matter of *d* but having slightly greater potency.

Similar separations with juices from canna roots, giant reed (*Arundo donax* L.) roots, gladiolus (*Gladiolus* sp.) corms, and Johnson grass (*Sorghum halepense* (L.) Pers.) roots are summarized in table 4. As with *Hemerocallis*, the potent material in the ether fraction was all removed by the aqueous sodium carbonate solution, from which it was recovered by acidification and ether extraction. The final fraction *e* was highly potent, only 0.0174 g of dry matter of canna material per 100 cc of final culture fluid being necessary to inhibit growth of *Phymatotrichum omnivorum*. With all these monocots, the potent materials in the ether extract proved completely soluble in 1-percent sodium carbonate solution.

TABLE 4.—*Inhibitory effect of fractions of juices from some monocotyledonous plants on growth of Phymatotrichum omnivorum*

Treatment of the fractions	Percent dry weight [1]	Growth (+) or lack of growth (−) in nutrient solution 70 plus the following concentrations of fractions—							
		12.0	9.0	6.0	3.0	1.5	1.0	0.75	0.25
Canna root juice:									
a. Complete juice, at pH 7.0							−	[2] −	++
b. Aqueous residue after ether extraction									
c. Complete ether extract	0.0100	−	−	−	−	++			
d. Residue from *c* after washing with sodium carbonate solution	.0020	++	++	++	++	++			
e. Ether extract from acidified washings	.0058	−	−	−	−	++			
Giant reed root juice:									
a. Complete juice, at pH 7.0							[3] Tr.	+	++
b. Aqueous residue after ether extraction					−	−		−	
c. Complete ether extract	.0333	−	−	−	+	++			
d. Residue from *c* after washing with sodium carbonate solution	.0195	+	+	+	++	++			
e. Ether extract from acidified washings	.0153	−	−	[2] −	++	++			
Gladiolus corm juice:									
a. Complete juice, at pH 7.0							−	−	−
b. Aqueous residue after ether extraction						−		−	−
c. Complete ether extract	.0395	−	−	−	++	++			
d. Residue from *c* after washing with sodium carbonate solution	.0212	++	++	++	++	++			
e. Ether extract from acidified washings	.0126	−	[2] −	[3] Tr.	++	++			
Johnson grass root juice:									
a. Complete juice, at pH 7.0							−	−	++
b. Aqueous residue after ether extraction				−	−	−		−	
c. Complete ether extract		−	−	−	[3] ++	[4] ++			
d. Residue from *c* after washing with sodium carbonate solution		++	++	++	++	++			
e. Ether extract from acidified washings		−	−	−	−	[4] ++			

[1] See footnote 1, table 2.
[2] Trace of growth in 1 tube only.
[3] Growth in 2 of 4 tubes.
[4] Abundant growth in 1 tube only.

TABLE 5.—*Inhibitory effect on growth of Phymatotrichum omnivorum of some fractions of onion juice, showing potency of washed ether extract*

Treatment of the fractions	Percent dry weight [1]	Growth (+) or lack of growth (−) in nutrient solution 70 plus the following concentration of fractions—							
		12.0	9.0	6.0	3.0	1.5	1.0	0.75	0.25
a. Complete juice, at pH 7.0							−	−	++
b. Complete ether extract	0.0344	−	−	−	−	−			
c. Residue from *b* after washing with 1-percent sodium carbonate solution	.0206	−	−	−	−	+			
d. Ether extract from acidified washings	.0091	−	−	−	+	++			

[1] See footnote 1, table 2.

Onion juice yielded somewhat different results (table 5). In several trials, a potent fraction was obtained by ether extraction of acidified sodium carbonate solution washings, just as with the other monocots tested. However, the onion fraction *c*, consisting of portions of the ether extract not soluble in aqueous sodium carbonate solution, was invariably highly potent also. Onion juice therefore contains, in addition to the potent sodium carbonate-soluble fraction apparently common to all the monocots tested, another potent ether-soluble fraction not removed by sodium carbonate solution. This explains why saponification yielded different results with onion and with corn

juices. The acidic fraction soluble in sodium carbonate solution was readily saponified in each case. This left with corn juice no potency in the nonsaponified fractions, while with the onions there remained the additional potent fraction, not soluble in sodium carbonate solution and only slowly saponifiable by potassium hydroxide.

SOME OTHER STUDIES ON ETHER FRACTIONS FROM MONOCOTYLEDONOUS PLANTS

COLD STORAGE OF ETHER FRACTIONS AS AFFECTING APPARENT POTENCY

Ether fractions from acidified sodium carbonate solution washings were stored on several occasions in stoppered flasks in the refrigerator. One of these was part of fraction *d* of table 5. After 8 months the dry weight of materials in this liquid had dropped to 0.0078 g per 100 cc of the original volume, presumably on account of a light brownish-white precipitate observed on the bottom of the flask. In cultures set up with this stored material, however, the potency was found to have increased so that no growth occurred at a concentration of 3.0. In the cultures shown in table 5, the addition of 0.0273 g of onion material per 100 cc of culture fluid had not inhibited growth, while the addition of a smaller dry weight of the stored material (0.0234 g) was now effective.

Similar results were obtained later with canna root material. The fresh juice was unusually low in potency. The ether extract from the sodium carbonate washings did not inhibit growth of *Phymatotrichum omnivorum* even at a concentration of 12.0, which provided 0.156 percent of plant material in the culture tubes. After storage for 8 days in the refrigerator, this fraction was again tested. The dry weight of the liquid had decreased slightly, but the potency had increased. A concentration of 10.0 provided 0.123 percent of plant material in the cultures, yet completely prevented growth of *P. omnivorum*.

In both cases, during storage of this fraction in the ether solution some change occurred which increased its potency. Oxidation or hydrolysis may have caused both the loss in weight and the increase in potency. The potent materials that prevent growth of *Phymatotrichum omnivorum* in the cultures are possibly produced by oxidation or hydrolysis and may be evidence of the existence of some precursor in the plant, and are not necessarily identical with the material as found in the original roots.

SOLUBILITIES OF THE SODIUM CARBONATE-SOLUBLE POTENT FRACTION

Further separation of potent fractions was attempted with ether extracts from several plants by layering with water plus alcohol or acetone. In general, more material and greater potency were obtained in ether-acetone, ether-methyl alcohol, and ether-ethyl alcohol fractions, and little material or potency in the corresponding aqueous acetone and aqueous alcohol fractions.

POTENCY AGAINST DIFFERENT STRAINS OF PHYMATOTRICHUM OMNIVORUM

Most of the experiments summarized here were performed with cultures of *P. omnivorum* strain 24, small portions of sclerotial masses being used as the inoculum. Several other sets of cultures were

set up with inoculum of strain 43 taken from agar slants. The results were the same, indicating that the reactions concerned are probably general for *P. omnivorum* rather than specific for a particular strain of the fungus.

TABLE 6.—*Inhibitory effect on growth of Phymatotrichum omnivorum of fractions of juices from some dicotyledonous plants*

Treatment of the fractions	Percent dry weight [1]	Growth (+) or lack of growth (−) in nutrient solution 70 plus the following concentrations of fractions—							
		12.0	9.0	6.0	3.0	1.5	1.0	0.75	0.25
Beet juice:									
a. Complete juice, at pH 7.0	------	------	------	------	------	------	+	++	++
b. Aqueous residue after ether extraction	------	−	−	−	−	++	------	------	------
c. Complete ether extract	0.0106	+	+	++	++	++	------	------	------
d. Residue from *c* after washing with sodium carbonate solution	.0073	+	+	+	++	++	------	------	------
e. Ether extract from acidified washings	.0035	++	++	++	++	++	------	------	------
Carrot juice:									
a. Complete juice, at pH 7.0	------	------	------	------	------	------	++	++	++
b. Aqueous residue after ether extraction	------	−	−	−	−	−	------	------	------
c. Complete ether extract	.0735	++	++	++	++	++	------	------	------
d. Residue from *c* after washing with sodium carbonate solution	.0701	++	++	++	++	++	------	------	------
e. Ether extract from acidified washings	.0032	++	++	++	++	++	------	------	------
Potato juice:									
a. Complete juice, at pH 7.0	------	------	------	------	------	------	Tr.	Tr.	+
b. Aqueous residue after ether extraction	------	−	−	−	−	++	------	------	------
c. Complete ether extract	.0109	−	Tr.	+	++	++	------	------	------
d. Residue from *c* after washing with sodium carbonate solution	.0031	++	++	++	++	++	------	------	------
e. Ether extract from acidified washings	.0080	−	Tr.	+	++	++	------	------	------
Sweetpotato juice:									
a. Complete juice, at pH 7.0	------	------	------	------	------	------	[2] +	+	++
b. Aqueous residue after ether extraction	------	------	------	−	−	−	------	------	------
c. Complete ether extract	.0305	Tr.	Tr.	+	++	++	------	------	------
d. Residue from *c* after washing with sodium carbonate solution	.0274	+	+	++	++	++	------	------	------
e. Ether extract from acidified washings	.0034	+	++	++	++	++	------	------	------
Turnip juice:									
a. Complete juice, at pH 7.0	------	------	------	------	------	------	−	−	[2] ++
b. Aqueous residue after ether extraction	------	−	−	−	−	−	------	------	------
c. Complete ether extract	.0194	−	−	−	−	−	------	------	------
d. Residue from *c* after washing with sodium carbonate solution	.0131	−	−	−	−	−	------	------	------
e. Ether extract from acidified washings	.0064	−	−	−	++	++	------	------	------

[1] See footnote 1, table 2.
[2] Growth in 2 of 5 tubes only.

COMPARATIVE STUDIES OF PREPARATIONS FROM JUICES OF SOME DICOTYLEDONOUS PLANTS

Since the sodium carbonate-soluble fraction from the ether extract occurred in all the monocots studied, it was of particular interest to find whether this potent fraction occurred also in dicots. Of the plants included in table 6, beets (*Beta vulgaris* L.), carrots, and sweetpotatoes are highly susceptible to root rot, while potatoes and turnips (*Brassica rapa* L.) are only moderately susceptible and belong to families that contain many resistant plants.

With all five plants, the aqueous residues after ether extraction (fraction *b*) were potent in preventing growth of *Phymatotrichum omnivorum*, as in earlier tests in which this fraction proved uniformly

potent. The ether fractions from the three more susceptible plants did not inhibit growth, while ether fractions from potato and turnip gave results similar to those obtained with monocots. With potato, the potency was entirely in the sodium carbonate-soluble fraction (*e*). In the present experiment all the turnip fractions were potent. This does not accord fully with results with another lot of turnip juice as given in table 1 but it does agree with the strong inhibiting effect of turnip juice noted in previous work (*3*).

The difference found here between the more susceptible and the more resistant dicots may be partly quantitative rather than qualitative, at least for the sodium carbonate-soluble fraction. A lower weight of this fraction was obtained from the susceptible dicots here than in corresponding fractions of potato or turnip juice, or of various monocots tested. These results suggest that differences between dicots in susceptibility to root rot may be based on differences in the content of certain ether-soluble materials, which may be of the same nature as those occurring in the immune monocots.

DISCUSSION

The work summarized in this paper has been essentially a gradual fractionation of plant juices, eliminating unimportant fractions by tests in which the root rot fungus itself served as the indicator. Disregarding unsuccessful and relatively unimportant steps, the present status of the fractionation is summarized graphically in figure 2.

Substances capable of inhibiting growth of *Phymatotrichum* in cultures were found among both ether-soluble materials and those remaining in the aqueous residue. The water-soluble portion (*b* of fig. 2) usually included at least 100 times as much of the plant material as the ether extract, and held back growth of the fungus in preparations from all plants studied, whether susceptible or immune. This portion has not yet been investigated extensively for the particular materials to which monocots owe their immunity.

More attention has been given to the ether extract, *c*, which was always potent in separations from monocots but not in those from susceptible dicots. Extraction with aqueous sodium carbonate solution further purified the ether-soluble potent material from the monocots, since materials going into fraction *d* were never potent, except with the onion. The onion contains what is apparently a special potent material, uniformly found in this fraction.

The washed ether extract *d* did not lack potency merely because of dilution. In one experiment with canna root juice, for example, this fraction with a dry weight of 0.0051 g per 100 cc was tested at a concentration of 48.0, making the culture solution contain 0.2448 percent of canna material. No inhibition of *Phymatotrichum* was obtained, although growth was inhibited with a tenth as much of some of the potent ether fractions.

The final fraction *e*, consisting of the potent material recovered from the sodium carbonate solution washings, was usually as potent as *c*, despite the lower dry weight. Except with onion, all the potent material of the ether extract of monocots was contained in this fraction. Relatively small amounts of this final fraction were required to inhibit growth of *Phymatotrichum omnivorum*. Growth was inhibited in cultures that contained less than 0.1 percent dry weight of the plant

material. The concentration necessary ranged from 0.0174 percent in a canna series to 0.0918 percent in a giant reed series.

Growth was prevented with complete canna juice diluted to about 2.25 percent, and with complete onion juice diluted to about 4.5 percent, of dry matter. With the final potent fractions of these juices, growth was prevented with only 0.0174 percent and 0.0546 percent, respectively, of plant material. Substances in these fractions were thus about 130 and 82 times as potent, for the same dry weight, as the original juices.

It may be questioned whether the same type of material was present in fraction *e* from some of the dicots. This fraction generally con-

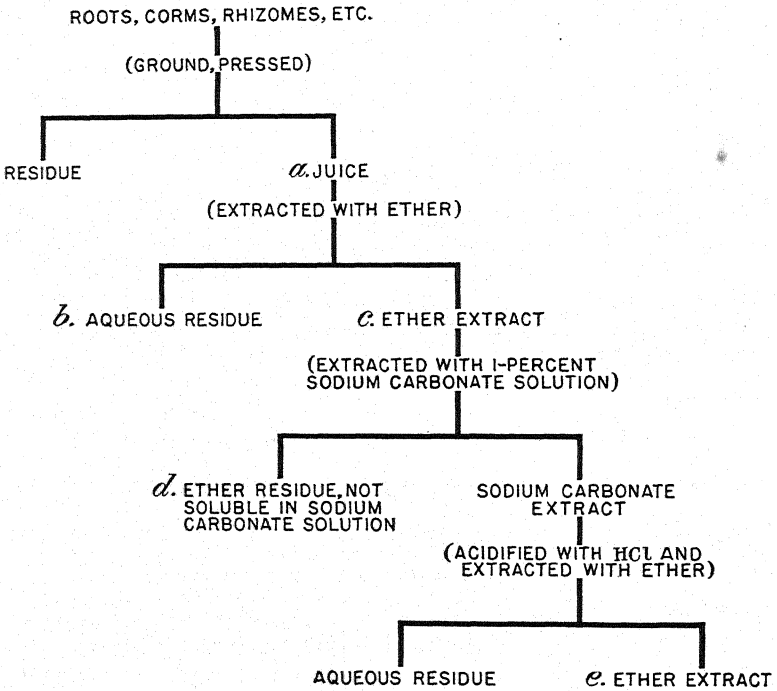


FIGURE 2.—Outline of fractionation of juices of monocotyledonous and dicotyledonous plants, as in experiments summarized in tables 3 to 6. Fractions tested are indicated here by the same letters as in tables 4 and 6.

tained little material. With the potato, a higher yield was obtained and growth of the fungus was inhibited in cultures containing 0.096 percent of the dry material. This may be the same type of material found to be nonpotent in the smaller amounts in other dicots. However, with the turnip a low weight of this fraction (only 0.0384 percent) in the cultures was sufficient to prevent growth of the fungus, which suggests that this turnip fraction might be qualitatively as well as quantitatively different from that of some of the other dicots. These fractions from some of the dicots may be associated with the resistance of various families and species to root rot.

The fractions tested were not exposed to conditions that might produce toxic byproducts not present in the original roots. The separations did not involve reagents likely to produce permanent

chemical changes in the plant materials. Fractionations with ether were followed always by washing with water to remove from the ether solution traces of water-soluble materials. Use of blanks in occasional experiments, and accompanying fractionations of dicot juices, showed that substances inhibiting growth in the cultures were actually from the monocot roots, and were not due to accumulations of traces of chemicals in the juices during manipulation.

By the methods used, roots of immune monocots all yielded an ether-soluble fraction capable of inhibiting the growth of the fungus, while roots of susceptible dicots did not. This fraction *e* (possibly in conjunction with some of the water-soluble inhibitory substances) is probably concerned in the immunity of monocots to root rot. It is then of some interest to consider the possible chemical nature of materials that may be in this fraction. The properties of this fraction are: Solubility in ether, from which it cannot be washed by water nor precipitated by acetone; solubility in aqueous sodium carbonate solution, from which it can be recovered by ether extraction after slight acidification; relative insolubility in petroleum ether as compared to ethyl ether; probable solubility in alcohols. This information is sufficient to exclude the bulkier plant constituents, sugars, proteins, and so on, which would be in the aqueous rather than in the ether fractions. It excludes phospholipins, which would be precipitated by the acetone. It excludes neutral fats or sterols, which would not go into the sodium carbonate solution. The potent fraction presumably consists of definitely acidic, ether-soluble substances, perhaps organic acids or esters.

SUMMARY

Root rot caused by *Phymatotrichum omnivorum* is known to attack 1,708 plants, all gymnosperms or dicots, while monocots are immune. Juices expressed from the roots of monocots contain some material that prevents growth of the fungus, which grows well in similar juice from susceptible dicots.

Ether extracts of juices from roots of monocots were potent in preventing growth of the fungus, while those from roots of susceptible dicots were not potent. The aqueous residues of juices from susceptible as well as from immune plants proved potent when tested in the culture solutions.

In ether extracts from all monocots tested, potent material has been found in a fraction characterized by the following properties: Solubility in ether, from which it cannot be washed by water nor precipitated by acetone; solubility in aqueous sodium carbonate solution, from which it is recovered in ether after slight acidification; relative insolubility in petroleum ether as compared to ethyl ether; probable solubility in alcohols. Fractions of this kind, which were approximately 100 times as potent on a dry-matter basis as the original juice, completely prevented growth of *Phymatotrichum omnivorum* when added to nutrient solutions in amounts that supplied from 0.02 percent to 0.09 percent of plant material. Such fractions were prepared from onion bulbs, gladiolus corms, and giant reed, canna, *Hemerocallis*, and Johnson grass roots.

Onion juice, which was studied in many experiments, was found to contain an additional potent fraction, insoluble in sodium carbonate

solution but apparently slowly saponifiable in alcoholic potassium hydroxide.

Ether fractions from susceptible dicotyledonous plants, such as carrots, beets, and sweetpotatoes, were uniformly nonpotent. However, the less susceptible potato and turnip yielded potent ether fractions. These fractions may be associated with differences in the susceptibility to root rot of various families and species of dicots.

The general immunity of monocots to root rot is due at least in part to the presence in the roots of these plants of minute quantities of acidic, ether-soluble substances, possibly organic acids or esters.

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A MEASURE OF TOXICITY IN PLANT STUDIES[1]

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The toxicity of a compound for a plant is usually expressed in terms of the minimum concentration of the compound required to kill the plant. Other standards of toxicity frequently used are the minimum concentration that prevents lengthening of the primary root of young

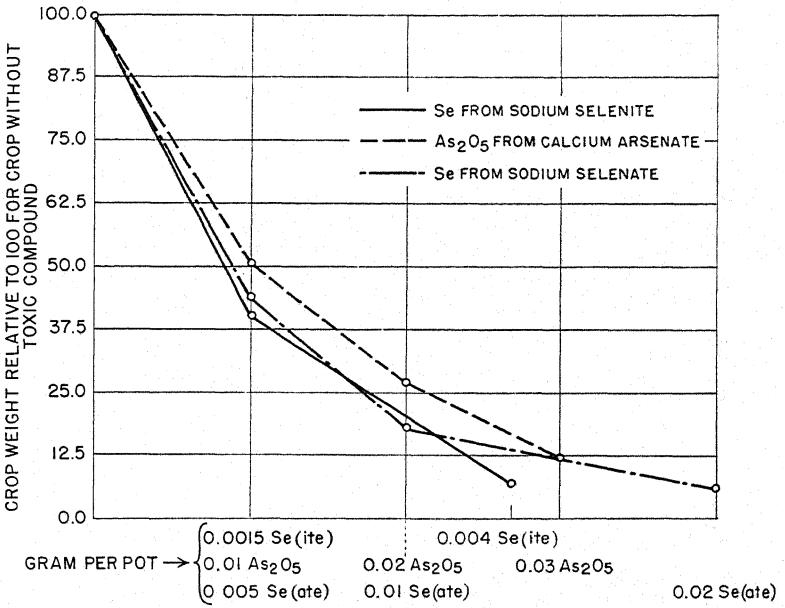


FIGURE 1.—Growth of millet in quartz sand with increasing quantities of calcium arsenate, sodium selenate, and sodium selenite.

seedlings and the concentration required to produce certain symptoms, such as chlorosis. Although these standards are adequate for many investigations, they are not entirely satisfactory for quantitative studies of toxicity, particularly where it is desired to measure the influence of some factor on toxicity. The end points are not sufficiently sharp.

The indefiniteness of the end point, concentration killing the plant, is shown by the curve for decreasing growth with increase in the toxic compound. Figure 1 shows how, in pot experiments, the growth of millet (*Setaria italica*) in quartz sand is affected by three toxic compounds—sodium selenate, sodium selenite, and calcium arsenate. The selenate curve represents the average of results obtained in 11 different experiments, except the point at 0.02 Se, which is a single determination. The selenite curve is an average of three experiments and the arsenate curve an average of nine experiments. All three curves are

[1] Received for publication September 16, 1937; issued June 1938.

of the same form. Probably this form holds for toxic substances in general when there is no interaction between the toxic substance and substances in the medium of growth. It is obvious from the gradual approach of the growth curve to the abscissa that a high order of experimental accuracy is needed to locate exactly the point of no growth (killing concentration).

A more satisfactory measure of toxicity is the quantity or concentration of toxic compound required to reduce growth one-half. This is a quite obvious criterion of toxicity that has been used by the writer [2] and probably by others. Apparently it should be in general use. The value is determined by growing plants with and without the toxic compound, plotting weight of crop against quantity of toxic compound, and locating the critical quantity of toxic compound from the curve. This measure of toxicity has a sharp end point. Figure 1 shows that at 50 percent of normal growth a small difference in the dose of a toxic compound produces a measurable difference in growth. Moreover, the toxicity curve shows that if the critical quantity of toxic compound is known approximately, the half value may be determined quite exactly by interpolation when only two or three differently treated lots of plants are grown. One lot of plants without the toxic compound and one or two lots with the compound are sufficient. Other advantages of the half value as a measure of toxicity are apparent in the data in table 1.

TABLE 1.—*Quantities of calcium arsenate, sodium selenate, and sodium selenite required to reduce growth of millet one-half in quartz sand, as determined at different times*

Calcium arsenate				Sodium selenate				Sodium selenite			
Date of planting	Duration of growth	Weight per pot of plants receiving no arsenate	Arsenate, as As_2O_5, required per pot to reduce growth one-half	Date of planting	Duration of growth	Weight per pot of plants receiving no selenate	Selenate, as Se, required per pot to reduce growth one-half	Date of planting	Duration of growth	Weight per pot of plants receiving no selenite	Selenite, as Se, required per pot to reduce growth one-half
	Days	*Grams*	*Gram*		*Days*	*Grams*	*Gram*		*Days*	*Grams*	*Gram*
Mar. 7	30	4.15	0.010	Jan. 14	42	3.60	0.0043	Sept. 1	23	3.16	0.0014
Mar. 24	31	3.29	.009	Feb. 11	35	3.12	.0054	Oct. 12	32	2.31	.0012
Apr. 17	25	2.67	.011	Feb. 27	35	2.09	.0041	Oct. 31	42	2.18	.0013
Apr. 21	26	2.74	.010	Apr. 1	30	2.90	.0038				
May 20	30	2.22	.011	May 24	25	4.03	.0042				
Aug. 8	25	3.74	.009	June 20	18	2.28	.0043				
Aug. 18	25	2.73	.009	Aug. 29	28	2.66	.0037				
Sept. 10	33	2.17	.012	Sept. 21	23	3.16	.0087				
Dec. 9	42	1.22	.012	Oct. 11	35	1.61	.0046				
				Oct. 12	32	2.31	.0053				
				Oct. 15	33	3.09	.0047				
Average			.010	Average			.0044	Average			.0013
Standard deviation			.0012	Standard deviation			.00056				
Coefficient of variability			12 percent	Coefficient of variability			13 percent				

Table 1 shows half-toxicity values obtained for calcium arsenate, sodium selenate, and sodium selenite in quartz sand with millet as the test crop. The nine determinations for calcium arsenate are replicates, except that they were made at different times of the year, and

[2] GILE, PHILIP L. THE EFFECT OF DIFFERENT COLLOIDAL SOIL MATERIALS ON THE TOXICITY OF CALCIUM ARSENATE TO MILLET. Jour. Agr. Research 52: 477-491, illus. 1936.

some of them in different years. This applies also to the 11 determinations for sodium selenate and the 3 for sodium selenite. It should be pointed out that these repetitions were not made with the idea of testing the accuracy of the toxicity determination. Millet was grown in pots in quartz sand, with and without a toxic compound, in a large number of experiments, to serve as a standard for comparing other data. It should also be understood that all determinations made in the course of several years are included in the table, except one obviously wrong, and that no attempt was made to secure the highest accuracy in these determinations. The plants were grown in 1-gallon pots and each treatment was in triplicate only.

The determinations of the toxicity values agree closely. This is surprising, for they were made at different times of the year when light and temperature conditions of the greenhouse varied markedly. The marked variability of growth conditions in general is shown by the comparative weights of the plants receiving no toxic compound in relation to the number of days the plants were grown. At different seasons the plants were grown for different lengths of time in order that they might be at approximately the same stage of maturity, the joint stage, when cut.

Apparently, this toxicity value is independent of ordinary variations in light and temperature. Since toxicity determinations, shown in table 1, have a variability of only 13 percent, differences in light and temperature, such as occur in a greenhouse from summer to winter, could not affect the half-toxicity value by more than this amount. Even this small variation is evidently not due to different growth conditions but to experimental error. For three toxicity values of sodium selenate had probable errors averaging ± 0.0006 g, as near as could be calculated; whereas the standard deviation for the sodium selenate values is ± 0.00056 g. In other words, the probable error of a determination accounts for the deviation from the average.

Prior to obtaining the above results it was thought that the toxicity value should vary with general growth conditions, for it seemed that the plants injured by arsenate or selenate should be affected differently from normal plants by changes in growth conditions. Evidently, however, normal plants and plants injured by arsenate or selenate have their growths altered in exactly the same proportion by changes in light and temperature. The toxicity values are, of course, not independent of all changes in growth conditions. Certain changes in nutrient salts, alteration in the sand medium, and variation in the method of applying the toxic compound may alter a toxicity value appreciably.

SUMMARY

It is suggested that the toxicity of a compound for a plant be measured by the quantity or concentration of the compound required to reduce growth by one-half.

This measure of toxicity can be determined with accuracy, since in the region of 50-percent reduction in growth a small difference in the dose of toxic compound produces a measurable difference in growth.

An advantage of this toxicity value is that it is independent of the marked differences in light and temperature that occur in an ordinary greenhouse between summer and winter.

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EFFECT OF PERCENTAGE OF PROTEIN IN THE DIET ON GROWTH AND FEED UTILIZATION OF MALE CHICKENS[1]

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INTRODUCTION

The optimum percentage of protein in the diet of the growing chick is of practical importance and scientific interest. Hence, a great deal of scattered research on the protein requirements for growth of chickens has been done with birds of different ages and breeds, under different systems of management, and fed protein from various sources. Even in some of the most carefully planned earlier studies of the protein requirements of chickens for growth, the significance of the results is doubtful in the light of present knowledge of the vitamins. It is probable that in some cases a part of the difference attributed to the level of protein intake was due to difference in vitamin G or mineral intake.

The experiment described in this paper was carried out for the purpose of obtaining extensive and reliable data on (1) the effect of the level of protein intake on growth of male chickens, utilization of feed, utilization of protein, and the relationship between feed consumption and live weight; and (2) the influence of limited feeding on the relationship between growth response and the level of protein intake. The experiment provided for (1) the limited feeding of seven diets containing different percentages of protein to seven lots of male chickens and the ad libitum feeding of the same diets to another seven lots of males, for a period of 52 weeks; (2) collecting the pertinent data on feed consumption, live weight, and mortality; and (3) making a mathematical analysis of the data. The limited-fed birds received 70 percent of the normal feed intake. Male chickens were used in this experiment so that the influence of egg production on feed consumption and growth would be avoided.

REVIEW OF LITERATURE

Papers on the protein requirements of growing chicks and on closely allied subjects have been published by many workers. Mussehl and Gish (*10*) [2] found that the rate of growth of chicks during the first 9 weeks was improved as the level of crude protein in the diet was increased to approximately 19 percent. Meat scrap was the chief source of protein in their diets. Holst (*6*) suggested that chicks should be fed a diet high in protein when first given feed and that the percentage of protein should be decreased progressively as the chicks advanced in age.

[1] Received for publication November 9, 1937; issued June, 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 810.

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Norris and Heuser (*11*) reported that chicks made the best growth during the first 8 weeks on a diet containing 20 percent of protein. Diets containing more than 20 percent were not studied. They also found that as the age of their birds increased the efficiency of protein utilization decreased, and that as the protein content of a diet increased the efficiency of feed utilization increased but in birds of the same age the efficiency of protein utilization decreased. Heuser and Norris (*4*) concluded that the growth of chicks is divided into three periods of about 6 to 7 weeks each and that during these three periods the following percentages of protein are the smallest that will produce rapid growth: 20 percent during the first period, 18 percent during the second, and 15 to 16 percent during the third.

Swift, Black, Voris, and Funk (*15*) found that the greatest gain in weight was obtained with a protein content of 22.9 percent in the diet and that the smallest gain was obtained with a protein content of 14.7 percent. Morris (*9*) reported that lots of chicks fed diets containing 14.32 to 23.13 percent of protein all consumed about the same quantity of feed but that the lots fed the higher percentage of protein grew best. Milne (*8*) obtained excellent growth with chicks fed diets containing 22.5 and 30.5 percent of protein during the first 6 weeks. When the protein was fed at a level of approximately 38.8 percent, growth was adversely affected after the third week. However, when the initial level of protein intake was reduced by decrements of 4.5 percent from 38.8 to 16.3 percent during the first 6 weeks, the chicks made approximately the same gain as those having a level of protein intake of 30.5 percent throughout the period. St. John, Carver, Johnson, and Brazie (*13*) found that 21 percent of protein gave the best early growth but was closely followed by approximately 17 percent. They found also that after the chicks were 10 weeks old a diet containing 13 percent of protein gave a satisfactory growth and that regardless of the level of protein intake the birds tended to reach the same live weight at 24 weeks of age.

Tepper, Charles, and Reed (*16*) reared chicks in batteries and found that 20 percent of protein, which was the highest level they studied, was the most efficient up to 13 weeks of age. Diets having a protein content of less than 17 percent were not found to be desirable for brooding chicks in batteries. Efficiency of feed utilization, based on gains in weight, decreased with advancing age. Birds on low protein levels matured more slowly both physically and sexually than those on higher levels of protein. Heuser and Norris (*5*) concluded that with the possible exception of birds fed diets extremely low in protein all birds of the same breeding finally reach about the same live weight. The strain they used reached about the same weights at 29 to 30 weeks when the quantity of protein consumed was sufficient to support growth. They believed that "mortality is probably affected where the protein level is very low and thus growth very much retarded." They stated that "mortality is possibly also greater where the protein level is excessively high, in which case there might probably be a deficiency of some other factor."

Wilgus, Norris, and Heuser (*18*) showed that the growth-promoting properties of the protein concentrates were, in general, due to their vitamin G content as well as to the quality of their protein. Norris, Heuser, Ringrose, Wilgus, and Heiman (*12*) presented evidence to show

that the requirements of chicks for vitamin G are extremely large. Heiman (*2*) has shown that 10 percent of dried whey insures ample vitamin G for growth.

Titus, Jull, and Hendricks (*17*) recommended that in comparative feeding experiments all the groups be fed a level of feed intake equal to 70 percent of an approximation of the idealized ad libitum feed consumption. They found that at the 70-percent level the value of the Spillman ratio was nearly as large as it was at the ad libitum level.

Carver, St. John, Miller, and Bearse (*1*) found that chicks fed skim-milk powder or Alaska herring fish meal at a 16-percent level of protein intake made better growth at 6 and 12 weeks of age than lots fed the same supplements at a 13-percent level. The pullets that received diets containing 13 and 16 percent of protein and in which the protein supplements were Alaska herring fish meal, meat scrap, or dried skim milk, showed very little difference in live weight at 24 weeks of age. Throughout the experiment, the pullets fed at a 13-percent level used the protein more efficiently than those fed at a 16-percent level. These investigators concluded that the protein requirements of pullets steadily decreased until the beginning of egg production.

McConachie, Graham, and Branion (*7*) found that the optimum protein content for growth in chicks during the first 6 weeks on the usual type of diet was approximately 25 percent. They concluded that for the first 12-week period approximately 19 percent of protein was the optimum amount and that a level of protein over 25 percent was detrimental to growth. These investigators reported that both high and low levels of protein intake, particularly the former, tended to destroy the barring and to change the contour and texture of the feathers. They also found "crow heads" to be associated with low protein intake and concluded that slipped tendons were not due primarily to a high protein intake per se.

EXPERIMENTAL MATERIAL AND METHODS

Approximately 1,100 cross-bred chicks were hatched in a commercial hatchery May 25, 1933, for use in this experiment. Barred Plymouth Rock females had been mated with Rhode Island Red males to obtain the advantage of the sex-linked barring factor for separating the sexes at hatching time. The male chicks arrived at the Agricultural Research Center, Beltsville, Md., where this experiment was carried on, early in the forenoon of May 26. They were weighed and the weak and otherwise unsuitable ones discarded. The 518 male chicks which remained were distributed among 14 pens so that each pen contained 37.

The chicks were brooded under electrically heated brooders in a series of pens, 11½ by 4 feet in size, in a hot-water-heated brooder house. They received their first feed and water when they were 1 day old. After they were 4 days old, they were allowed access to direct sunlight in concrete yards, 12½ by 4 feet in size, adjoining their pens.

When the birds were 12 weeks old, it was necessary to reduce the number per lot to 28 to avoid overcrowding. At 20 weeks the number in each lot was further reduced to 20 birds. In each instance the reduction was made in a manner that would not change the mean live weight of the lot.

THE DIETS

The diets fed in this experiment were made up of combinations of the feed mixtures shown in table 1.

TABLE 1.—*Feed mixtures used in the experimental diets*

Ingredient	High-protein feed mixture [1]	Low-protein feed mixture [2]	Ingredient	High-protein feed mixture [1]	Low-protein feed mixture [2]
	Percent	*Percent*		*Percent*	*Percent*
Wheat gray shorts	25.0	25.0	Wheat-germ meal	2.5	2.5
Ground yellow corn	20.0	37.4	Ground gypsum	2.0	2.0
Granulated casein	15.0	0	Ground limestone	2.0	2.0
Dried whey	10.0	10.0	Common salt	.5	.6
Rolled oats	10.0	10.0	Monocalcium phosphate	0	.3
Alfalfa leaf meal	5.0	5.0	Dicalcium phosphate	0	.2
Wheat bran	5.0	5.0			
Desiccated meat meal	3.0	0	Total	100.0	100.0

[1] Average crude protein content, 26.54 percent.
[2] Average crude protein content, 12.87 percent.

The percentage of crude protein in each batch of the feed mixtures was determined before they were combined to form the diets. Then the two feed mixtures and 2 percent of cod-liver oil were combined to form the seven diets, the average chemical composition of which is given in table 2. The chemical composition of the diets differed significantly only in protein and nitrogen-free extract. All the vitamins and mineral elements known to be essential in the nutrition of the chicken were present in adequate quantities, and the quantities of each in the several diets were approximately equal.

TABLE 2.—*Average proximate chemical analyses of the experimental diets*

Diet No.	Crude protein (6.25 × total N)	Nitrogen-free extract	Fat (ether extract)	Crude fiber	Ash	Moisture	True protein (6.25 × albuminoid N)	Non-protein nitrogen compounds	Calcium (Ca)	Phosphorus (P)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	25.00	49.92	5.48	3.26	6.64	9.70	23.17	1.37	1.36	0.61
2	23.00	51.73	5.53	3.29	6.67	9.78	21.26	1.31	1.37	.61
3	21.00	53.54	5.58	3.32	6.70	9.86	19.36	1.24	1.37	.60
4	19.00	55.36	5.62	3.35	6.73	9.94	17.45	1.16	1.37	.60
5	17.00	57.18	5.67	3.38	6.76	10.01	15.55	1.09	1.37	.60
6	15.00	58.99	5.72	3.41	6.79	10.09	13.64	1.02	1.37	.60
7	13.00	60.80	5.77	3.44	6.82	10.17	11.74	.95	1.37	.60

These diets were fed to 14 lots of male birds, 7 of which were fed ad libitum and 7 of which were fed at a limited level approximating 70 percent of the idealized normal level of feed intake. The daily feed allowance for the limited-fed lots was calculated from the data compiled by Titus, Jull, and Hendricks (*17*) for males of this same cross.

Records were kept of the live weights of the chicks at the end of each week, of the quantities of feed consumed per chick per week, and of the mortality in each pen. The great volume of data compiled makes their complete publication impractical, hence only representative data are presented. The ages of the birds for which the data are presented are those at which cockerels are ordinarily marketed.

EXPERIMENTAL DATA

MORTALITY

The data in table 3 show the mortality by periods for the several lots. During the first 8 weeks the mortality was greater among the limited-fed birds than among those consuming their feed ad libitum. It was also observed that during this period there was a tendency for the mortality to be greater on the two lower levels of protein intake than on the higher levels. During the last 30 weeks of the experiment, mortality was due to scattered cases of neurolymphomatosis and injuries received in fights.

TABLE 3.—*Mortality of birds on the various diets during the periods indicated* [1]

Diet No.	Crude protein in diet	Birds that died during period from—							
		0 to 8 weeks	8 to 12 weeks	12 to 16 weeks	16 to 20 weeks	20 to 30 weeks	30 to 40 weeks	40 to 52 weeks	Total
	Percent	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
LOTS FED AT 70 PERCENT OF NORMAL FEED INTAKE									
1	25	2	0	0	0	1	0	2+(2)	5+(2)
2	23	3	1	0	0	0	3	2+(2)	9+(2)
3	21	5	1	0	0	0	0	1+(1)	7+(1)
4	19	4	1	0	0	1	2	2+(2)	10+(2)
5	17	3	1	0	0	1	0	(2)	5+(2)
6	15	5	2	0	0	1	1	(4)	9+(4)
7	13	7	0	2	0	1	1+(1)	5	16+(1)
LOTS FED AD LIBITUM									
1	25	0	0	0	0	0	1	1+(2)	2+(2)
2	23	3	0	1	0	1	1+(2)	2+(2)	8+(4)
3	21	0	0	0	0	0	3+(1)	1+(3)	4+(4)
4	19	2	0	0	2	2	0	2+(2)	8+(2)
5	17	2	0	0	0	0	3	1+(3)	6+(3)
6	15	4	0	0	1	0	1+(1)	2+(1)	8+(2)
7	13	4	0	1	1	2	2	2+(1)	12+(1)

[1] Numbers in parentheses indicate birds that were killed by other birds.

LIVE WEIGHTS

The mean live weights of the birds in the several lots, together with the standard errors of the means, are given in table 4. The data show that at all ages the birds fed ad libitum weighed more than those fed the same diet with the intake limited to 70 percent of the normal. At 8 weeks of age, and through the sixteenth week, the weights of the birds in both groups that received 17 percent of protein in their diets were greater than the weights of birds that received 15 percent, and the birds that received 15 percent of protein weighed more than those that received 13 percent. At 16 weeks of age the birds that received 25 and 23 percent of protein were heavier than those that received 19 and 17 percent, which in turn were heavier than those that received 15 and 13 percent. The difference in live weight between the birds that received 13 percent of protein and those in the other lots was the greatest at this time. At 20 weeks of age the live weight of the birds remained in nearly the same relative order as at 16 weeks. By the end of the thirtieth week all the lots fed ad libitum, except the one receiving the diet containing 13 percent of

protein, had about the same average live weight. In the limited-fed lots differences in live weight in favor of the higher levels of protein intake persisted through the thirtieth week, but by the end of the experiment all the lots had approximately the same average live weight. By the end of the fortieth week, the birds on the lower levels of protein intake in the lots fed ad libitum had attained practically the same average live weights as those on the higher levels of protein intake.

TABLE 4.—*Mean live weights, and their standard errors, of birds on the various diets at the ages indicated*

Diet No.	Protein in diet	Mean live weight at end of—						
		8 weeks	12 weeks	16 weeks	20 weeks	30 weeks	40 weeks	52 weeks
	Percent	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
LOTS FED AT 70 PERCENT OF NORMAL FEED INTAKE								
1	25	600±24	1,161±38	1,709±36	2,038±41	2,574±67	2,837±72	2,761±73
2	23	570±25	1,074±39	1,608±38	1,973±42	2,428±69	2,571±75	2,743±76
3	21	573±22	1,078±33	1,611±32	1,896±36	2,409±59	2,657±63	2,639±65
4	19	535±24	986±38	1,470±36	1,815±41	2,304±61	2,544±72	2,584±73
5	17	570±22	1,010±34	1,480±33	1,885±37	2,377±61	2,631±65	2,639±67
6	15	490±24	917±38	1,403±36	1,775±41	2,338±61	2,711±72	2,740±73
7	13	390±26	710±41	1,089±39	1,484±44	2,244±72	2,536±78	2,707±79
LOTS FED AD LIBITUM								
1	25	669±23	1,256±35	1,893±34	2,318±38	3,088±62	3,142±67	3,104±69
2	23	678±26	1,227±41	1,862±39	2,256±44	3,001±72	3,241±78	3,111±79
3	21	651±28	1,121±42	1,734±41	2,287±46	3,094±77	3,120±81	3,054±83
4	19	599±24	1,135±36	1,777±35	2,261±39	3,034±65	3,085±70	2,967±71
5	17	655±26	1,193±41	1,692±39	2,010±44	3,067±72	3,105±78	3,004±79
6	15	559±24	1,092±36	1,637±35	2,041±39	3,004±66	3,141±70	3,000±71
7	13	467±25	773±39	1,160±38	1,672±42	2,724±69	3,110±75	3,024±76

Although the data in table 4 show that there were appreciable differences in mean live weight among the lots that received the diets of higher protein content, in many instances these differences were not statistically significant. However, at the age of 8 weeks and through the thirtieth week, there were statistically significant differences between the lots fed high- and those fed low-protein diets, regardless of whether the feed intake of the birds was limited or not.

FEED CONSUMPTION

The average cumulative feed consumption of thebirds at representative ages is given in table 5. In the lots fed ad libitum, the birds receiving the highest percentage of protein ate the most feed per week during the first 30 weeks of the experiment. During the last 22 weeks there was no consistent relationship between the protein content of the diet and the quantity of feed eaten by any of the lots of the ad libitum group.

TABLE 5.—*Cumulative feed consumption per bird on the various diets at the ages indicated*

Diet No.	Protein in diet	Average feed consumption during—						
		0 to 8 weeks	0 to 12 weeks	0 to 16 weeks	0 to 20 weeks	0 to 30 weeks	0 to 40 weeks	0 to 52 weeks
	Percent	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
LOTS FED AT 70 PERCENT OF NORMAL FEED INTAKE								
1	25	1,463	3,097	5,170	7,495	13,788	20,340	28,224
2	23							
3	21							
4	19							
5	17							
6	15							
7	13							
LOTS FED AD LIBITUM								
1	25	2,553	4,855	9,166	12,625	21,469	30,624	42,303
2	23	2,378	4,442	7,448	9,934	18,918	29,098	42,673
3	21	1,822	3,662	6,714	9,523	18,945	29,441	42,835
4	19	1,942	3,989	6,784	9,508	18,948	27,758	38,923
5	17	2,057	4,424	7,088	9,706	18,715	28,318	40,697
6	15	1,874	4,162	6,959	9,570	18,251	27,331	38,125
7	13	1,803	3,281	5,878	8,718	18,112	28,105	40,741

In contrast to the findings of McConachie and others (*7*), no abnormality in contour, texture, or barring of feathers was found in any lot, regardless of the level of protein intake. No bird in this experiment showed any symptoms of perosis.

ANALYSIS OF THE DATA

Trends in the growth of the birds and in the utilization of feed and protein are revealed more clearly by plotting the data after they have been smoothed by the method of moving averages. Figure 1 shows the smoothed average live weights, plotted against age in weeks, of the limited-fed lots and those fed ad libitum. In figures 1, 2, and 3 the curves for the lots fed diets containing 19 and 23 percent of protein have been omitted to prevent crowding the charts.

In the group that was fed ad libitum, as well as in the group that received only 70 percent of the normal level of feed intake, the higher the percentage of protein in the diets the greater were the average live weights of the birds up to the time they reached mature weight. The birds reached mature weight in the inverse order of the quantity of protein they had consumed. In each group the lots receiving 17 percent or more of protein did not differ markedly from one another but showed a distinct advantage over those receiving 15 percent of protein and a very pronounced advantage over those receiving 13 percent of protein. On all diets the birds fed at the limited level of feed intake ceased to grow when they were approximately 88 percent as heavy as those fed the same diets ad libitum.

The live weight of the chicks fed ad libitum were in the same order as the protein contents of their diets during the first 14 weeks of age. With the exception of the two lots fed 13 percent of protein in the diet, the gains per week of the chicks fed ad libitum and those fed a limited diet increased until the fourteenth week and then gradually declined until the forty-fourth week. The weekly gains of the two lots receiv-

ing 13 percent of protein did not reach a maximum until the seventeenth week, but the birds in these two lots gained more weight between the seventeenth and forty-fourth weeks than any other lots of their respective groups. Although their gains were not so uniform, the birds in the group fed ad libitum gained more rapidly in all cases than the birds fed the same percentage of protein in the limited-fed group. During the first 14 weeks it was found that the magnitude of the

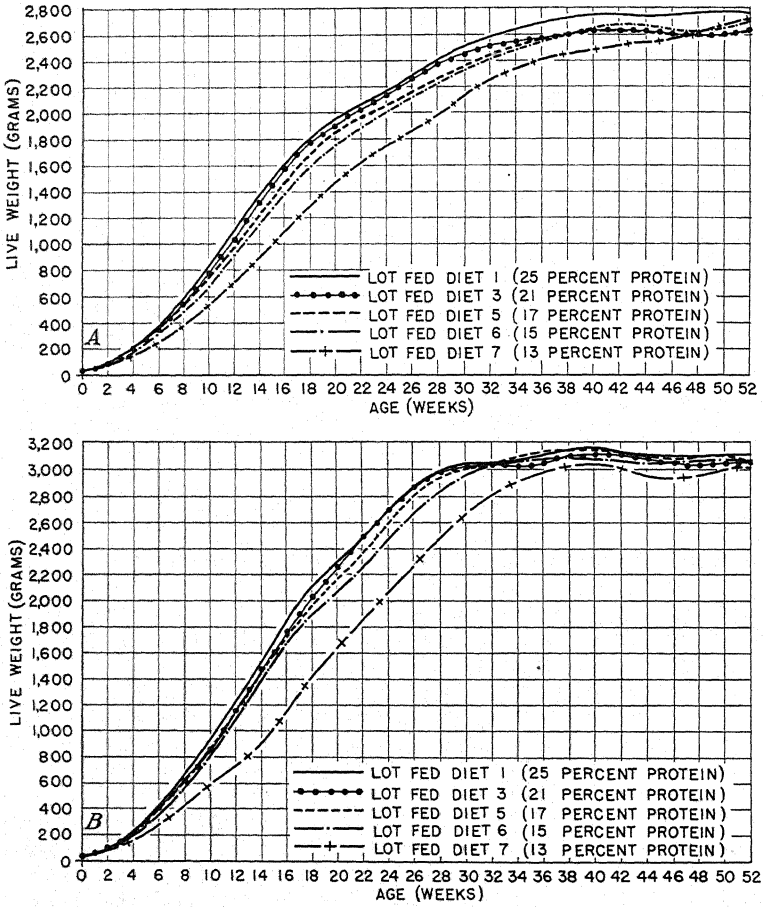


FIGURE 1.—Comparison of live weights of chickens, plotted against age, of the limited-fed (*A*) and of the ad libitum-fed (*B*) lots receiving various levels of protein in their diets. (Data smoothed by method of moving averages.)

average weekly gain in both groups was closely associated with the quantity of protein consumed; however, after the fourteenth week it was much less so.

The relative rates of gain were plotted according to the method used by Heuser and Norris (*4*) and compared with their published curves. The resulting curves resembled inverted curves of diminishing increment. The relative rates of gain reached zero at about the forty-fourth

week. The only irregularity was exhibited by the curve for the lot fed, ad libitum, the diet containing 13 percent of protein. The relative rates of gain for this lot showed a slight rise from the twelfth to the eighteenth week and a more gradual decline thereafter. The chief differences between diets were that during the first 10 weeks the higher the percentage of protein in the diet the higher the relative rate of gain and after the twentieth week the higher the percentage of protein in the diet the lower the relative rate of gain. The birds on limited feed made lower relative rates of gain during the first 2 weeks than those fed, the same diets ad libitum. Thereafter the relative rates of gain for birds on limited and on ad libitum feed of the same percentage of protein were almost identical. This indicates that as long as the diet is adequate for growth the type of curve followed by the relative rates of gain is not changed by the percentage of protein in the diet fed.

When average live weights are plotted against cumulative feed consumption, the resulting curves for the lots on limited-feed intake are one above another in the order of increasing percentage of protein in the diet, as shown by figure 2, *A*. However, figure 2, *B*, shows that when the same diets were fed ad libitum, the curves are one above another for the lots fed diets containing from 13 to 21 percent of protein, but the data show that the curve for the lot receiving 25 percent of protein would be below that for the lot receiving 21 percent of protein. Furthermore, the curve for the birds receiving 25 percent of protein is between those for lots receiving 15 and 13 percent of protein.

When average live weight was plotted against cumulative protein consumption (fig. 3, *A*, *B*), it became evident that the most efficient utilization of protein was by the birds receiving 15 percent of protein in each group. The figures indicate that the lots fed 15 percent of protein were followed, in the order of decreasing efficiency of protein utilization, by those that received 17, 13, 21, and 25 percent of protein in their diets. However, in the group fed a limited diet, the data in the next to the last column of table 6 indicate that the lot that received a diet containing 17 percent of protein was slightly more efficient in the utilization of protein than was the lot that received a diet containing 15 percent. This is also shown in figure 5, *A*. In the group fed ad libitum, the lot that received 25 percent of protein was strikingly less efficient in its use, in spite of its very rapid growth.

CALCULATED PARAMETERS AND CONSTANTS

The equation of the curve of diminishing increment may be written

$$W=A-Be^{-kF} \tag{1}$$

The first derivative of this equation is

$$\frac{dW}{dF}=kA-kW \tag{2}$$

If $\frac{dW}{dF}$ is defined as the efficiency of feed utilization, E, and if C is written in place of kA, the first derivative becomes

$$E=C-kW \tag{3}$$

which is the equation of a straight line.

A represents the maximum live weight attainable on a given diet at a given level of intake; B is equal to A minus the weight at the time of first feeding, and hence represents the maximum gain attainable; C is the gain in live weight that a unit of feed could produce if no feed were required for maintenance; k is the rate of decrease in efficiency.

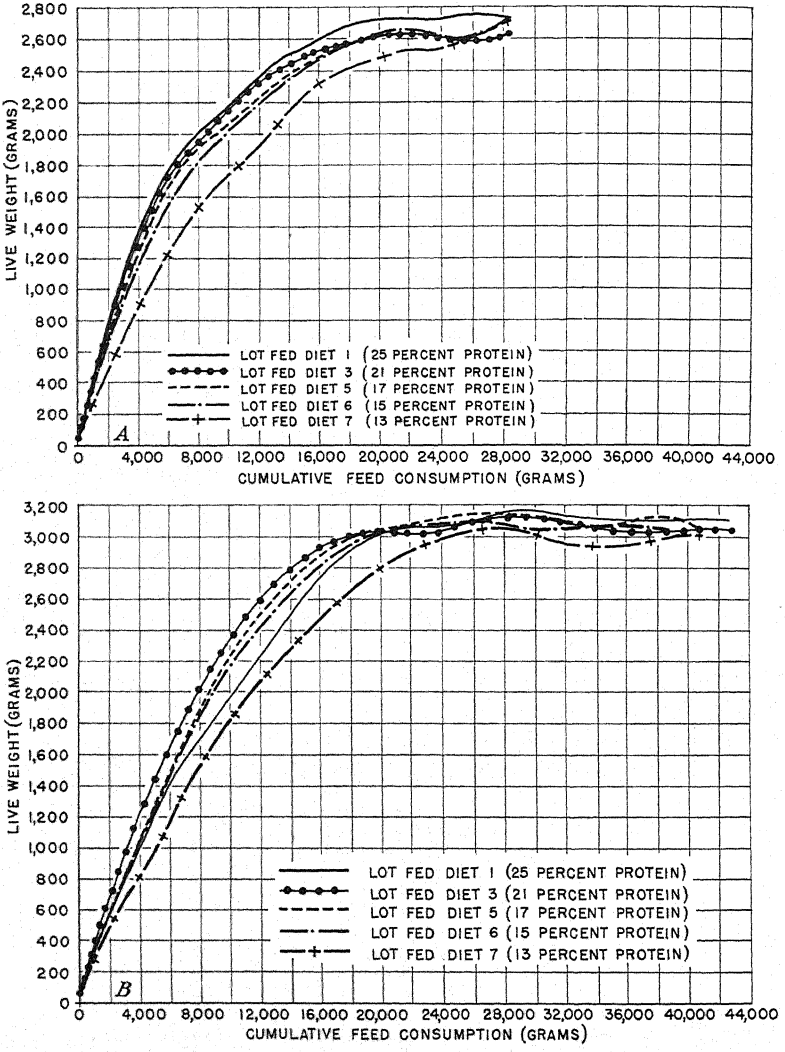


FIGURE 2.—Comparison of live weights of chickens, plotted against cumulative feed consumption, of the limited-fed (*A*) and of the ad libitum-fed (*B*) lots receiving various levels of protein in their diets. (Data smoothed by the method of moving averages.)

In other words, k is the amount by which the efficiency is decreased as the chicken gains one unit (1 g in this paper) of live weight. The quantity kW represents the loss in efficiency due to the maintenance requirement.

If E is divided by the percentage of protein in the diet, the result is the efficiency of protein utilization. Likewise if C is divided by the percentage of protein in the diet, the result (next to last column of table 6) is the gain in live weight that a unit of protein could produce if none were used for maintenance.

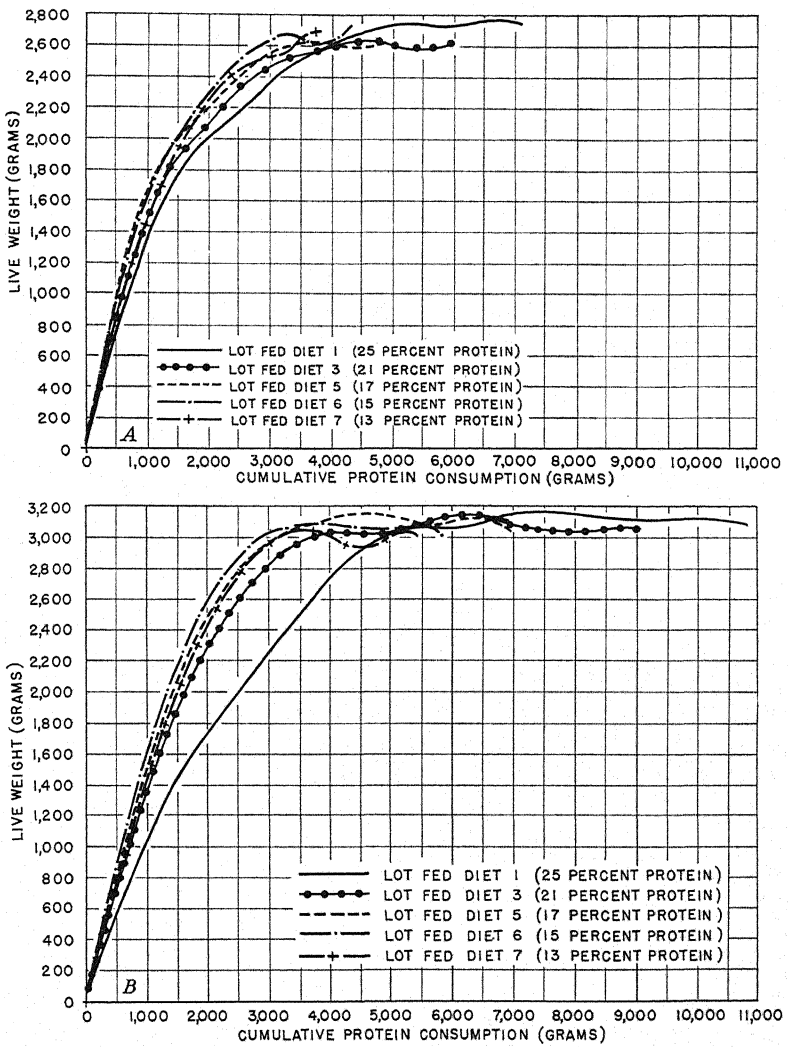


FIGURE 3.—Comparison of live weights of chickens, plotted against cumulative protein consumption, of the limited-fed (*A*) and of the ad libitum-fed (*B*) lots receiving various levels of protein in their diets. (Data smoothed by the method of moving averages.)

When $F=0$, E in equation (3) is equal to kB; that is to say, kB is the efficiency at first feeding, or the initial efficiency. R, the Spillman ratio, is the numerical value of e^{-k}, and is equal to the inverse ratio of the gain in live weight resulting from any two successive units of feed consumed.

The values of A, B, and k were determined by the method described by Hendricks (*3*). The adjustments suggested by Titus, Jull, and Hendricks (*17*) made no appreciable change in the values of the parameters when applied to these data and hence were not used. The numerical results of fitting equation (3) to the data on live weight and feed consumption are presented in table 6.

TABLE 6.—*Values of the parameters and derived constants of the live-weight and feed-consumption equations*

Diet No.	Protein in diet	A	Standard error of A	B	C	Standard error of C	1,000 k	Standard error of 1,000 k	R[1]	Standard error of R	kB	C	Standard error of C
												Percentage of protein	Percentage of protein
	Percent	*Grams*	*Grams*	*Grams*									
LOTS FED AT 70 PERCENT OF NORMAL FEED INTAKE													
1	25	2,809	±85	2,771	0.4586	±0.0235	0.16329	±0.01098	0.999837	±0.000011	0.4525	1.8344	±0.0940
2	23	2,742	±78	2,702	.4417	±.0211	.16107	±.01015	.999839	±.000010	.4349	1.9204	±.0916
3	21	2,703	±81	2,664	.4316	±.0217	.15966	±.01057	.999840	±.000011	.4253	2.0552	±.1035
4	19	2,640	±88	2,602	.4191	±.0231	.15872	±.01153	.999841	±.000011	.4130	2.2058	±.1214
5	17	2,690	±74	2,652	.4129	±.0185	.15349	±.00916	.999846	±.000009	.4070	2.4288	±.1088
6	15	2,816	±111	2,778	.3604	±.0211	.12799	±.01046	.999872	±.000010	.3556	2.4027	±.1403
7	13	2,897	±194	2,858	.2767	±.0222	.09551	±.01192	.999904	±.000012	.2730	2.1285	±.1705
LOTS FED AD LIBITUM													
1	25	3,314	±119	3,276	0.3313	±0.0197	0.09996	±0.00786	0.999900	±0.000008	0.3275	1.3252	±0.0786
2	23	3,308	±119	3,269	.3544	±.0210	.10712	±.00840	.999893	±.000008	.3509	1.5408	±.0911
3	21	3,222	±95	3,185	.3895	±.0191	.12087	±.00776	.999879	±.000008	.3850	1.8548	±.0907
4	19	3,182	±121	3,144	.3636	±.0227	.11428	±.00946	.999886	±.000009	.3593	1.9137	±.1193
5	17	3,259	±222	3,221	.3656	±.0400	.11217	±.01642	.999888	±.000009	.3613	2.1506	±.2353
6	15	3,237	±158	3,198	.3406	±.0260	.10523	±.01081	.999895	±.000016	.3365	2.2707	±.1731
7	13	3,281	±148	3,243	.2659	±.0156	.08105	±.00696	.999910	±.000007	.2628	2.0454	±.1202

[1] The corresponding values, when the unit of feed consumption and live weight is 1 kg. are 0.8493, 0.8512, 0.8524, 0.8532, 0.8577, 0.8692, and 0.9059 for the lots fed at 70 percent of the normal feed intake, and 0.9049, 0.8984, 0.8862, 0.8920, 0.8939, 0.9001, and 0.9222 for the lots fed ad libitum.

Figure 4 illustrates, for each lot of the limited-fed and the ad libitum-fed groups, respectively, the linear relationship between E and live

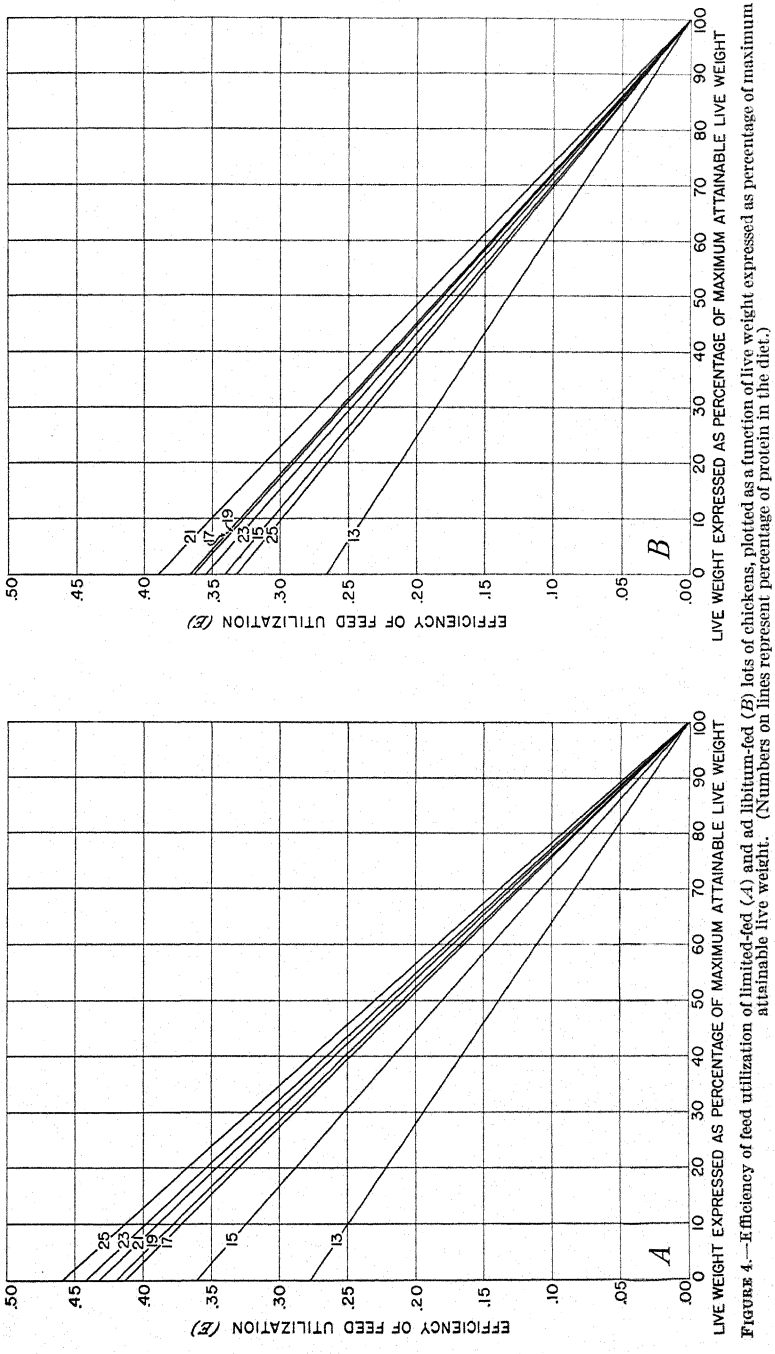


FIGURE 4.—Efficiency of feed utilization of limited-fed (*A*) and ad libitum-fed (*B*) lots of chickens, plotted as a function of live weight expressed as percentage of maximum attainable live weight. (Numbers on lines represent percentage of protein in the diet.)

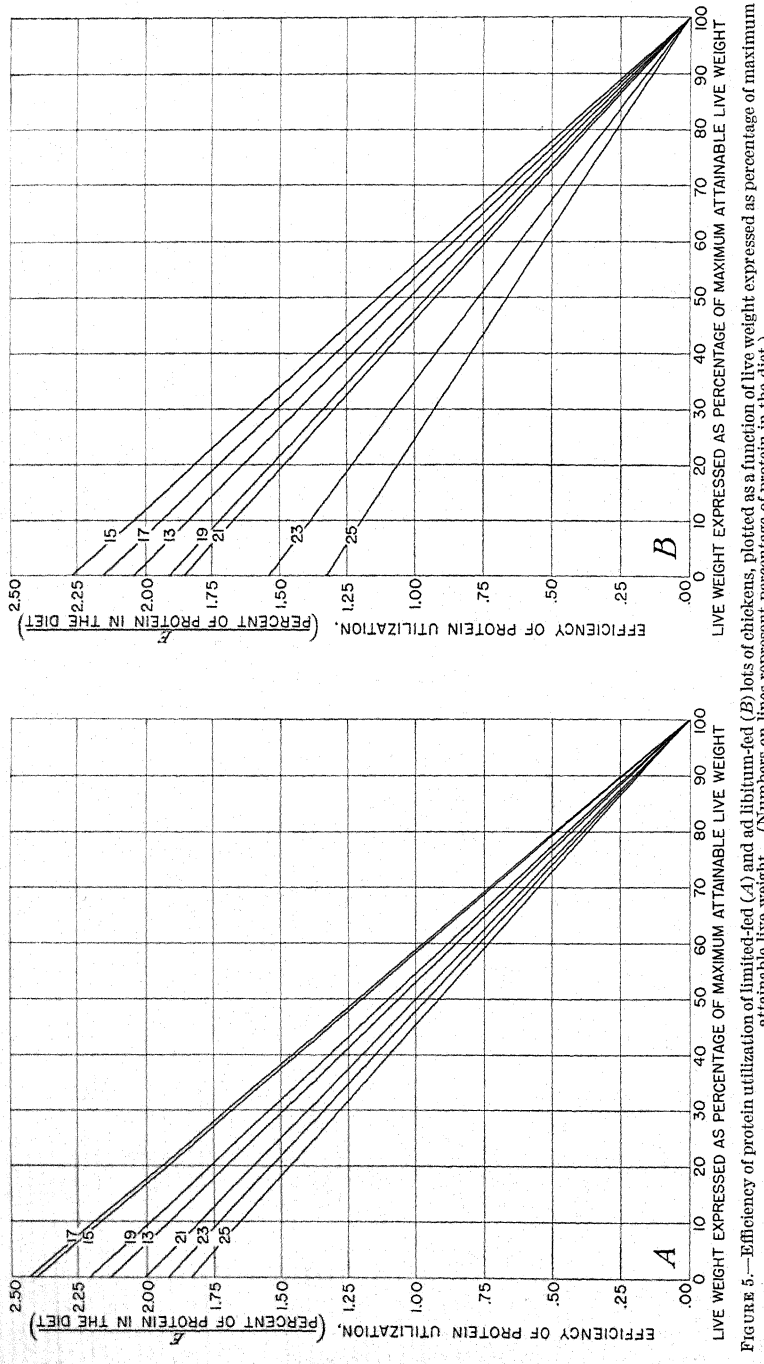


FIGURE 5.—Efficiency of protein utilization of limited-fed (*A*) and ad libitum-fed (*B*) lots of chickens, plotted as a function of live weight expressed as percentage of maximum attainable live weight. (Numbers on lines represent percentage of protein in the diet.)

weight expressed as percentage of A. The use of the percentages of maximum attainable live weight as abscissas in these figures is permissible because, as is indicated by the standard errors of A in table 6, no significant difference was found between the mature weights of any two lots in either group. However, the average value of A for the lots fed at 70 percent of the normal feed intake is significantly less than that for the lots fed ad libitum, and this difference should be kept in mind in comparing any lot in one group with any lot in the other group. In any case, the common point of zero efficiency established by using the percentage of maximum live weight instead of maximum live weight itself helps to show more simply the difference in efficiency of the lots within each group. Figure 5 illustrates a similar linear relationship between values of

$$\frac{E}{\text{percentage of protein in the diet}}$$

and the percentage of the maximum live weight. Figures 4 and 5 demonstrate that the efficiencies of feed or protein utilization at any given live weight are in the same relative order that they are at the initial live weight.

DISCUSSION

Mortality, which at times is the only clue to unknown nutritional deficiencies, was significantly associated with nutrition but twice in this experiment. In one instance limitation of the intake of feed (energy) was the cause; in the other, limitation of the quantity of protein.

Up to the time that the birds in each lot reached a mean live weight of 1,500 g, the birds in the limited-fed lots used their feed more efficiently than those fed the same diets ad libitum. However, the limited-fed lots required more time than the ad libitum-fed lots to reach any given mean live weight.

At the end of the experiment, the birds that had been fed at the 70-percent level of normal feed intake weighed, on an average, 88 percent as much as those fed the same diet ad libitum. Their feed consumption averaged 69 percent of that of the birds fed ad libitum, and thus their gross efficiency of feed utilization for the experiment was higher than that of the birds fed the same diets ad libitum. However, the birds in the group fed ad libitum reached an average weight of 2,700 g at 26 weeks of age, at which time they had eaten an average of 14,600 g of feed. The birds in the limited-fed group did not reach an average weight of 2,700 g until 48 weeks of age, by which time they had eaten an average of 26,300 g of feed. Hence, in spite of the higher gross efficiency of feed utilization of the limited-fed group, the birds fed ad libitum attained a weight of 2,700 almost twice as efficiently, in respect to feed consumed, as the limited-fed group. Obviously this was due to the fact that the birds in the limited-fed group had to be maintained 22 weeks longer than those in the other group before the average of 2,700 g was reached. This instance is an example of the fallacies that may be derived from reliable data when efficiencies of feed or protein utilization are compared at a given age.

As shown in table 5, during the first 16 weeks the two lots that were fed, ad libitum, diets containing 25 and 23 percent of protein—especially the one receiving 25 percent—ate much larger quantities of feed than any of the other lots. Since they grew but little faster than the lot fed 21 percent of protein, it seems quite possible that the high protein intake stimulated their appetites to such an extent that they consumed more feed than they could digest and absorb. Unfortunately, there is at present no definite way of testing the validity of this explanation because of the lack of information about the nitrogen metabolism of birds fed high protein diets ad libitum. It is worthy of note that according to the smoothed average-live-weight curves (fig. 1A) the birds in these two lots averaged more in live weight at all ages to maturity than did the birds in any of the more efficient lots. Thus, if rapid growth were the only consideration, the diets would increase in value in the order of their protein content, at least between the limits of 13 and 25 percent of protein. When feed costs must be considered, the use of 25 and 23 percent of protein fed ad libitum would not be justified on the basis of this experiment.

Since the values of C were derived by fitting the equation of the first derivative of the curve of diminishing increment to the observed data on feed consumption and live weight, it was possible to estimate the standard errors of these values of C. Examination of the standard errors of the values of C shows that, although there is a definite and regular upward trend in the value of C with each increment of protein for the limited diets, the differences are not statistically significant except between the two lowest levels of protein intake and between each of the two lowest levels and all others. In the lots fed ad libitum the value of C tended to increase as the protein in the diet increased from 13 to 21 percent, and then to decrease. In these same lots there were significant differences between the values of C for the following levels of protein intake: 21 and 25, 21 and 13, and 15 and 13. The deviation of C for the 19-percent level from its logical position between the values of C for the 17- and 21-percent levels is far from being statistically significant.

Since C represents the maximum value that E can take, the point at 0 live weight on each line in figure 4, A and B, is the C for that lot. From figure 4 it can be seen that at any specified live weight the efficiencies of feed utilization of a series of diets are in exactly the same relative position as the values of C, the maximum theoretical efficiency. This point is important since the erroneous impression is frequently gained, when efficiencies are plotted against age, that the lower protein diets are used more efficiently than the high protein diets in the later phases of growth. The reason for this impression is that birds on low protein diets usually do show more efficient use of feed at any given age after about the sixteenth week until their growth is completed. However, the fallacy in the reasoning is that, although the birds compared are of the same age, they are of different live weights and are in different phases of growth. When the birds on the low protein diets reach the same live weights as those with which they are compared on an age basis, it is found that they use their feed even less efficiently to gain any specified percentage of their maximum live weight.

In actual practice, these findings may not be of great importance, because the slight loss in efficiency resulting from feeding a lower protein diet during the later phases of growth may be offset by a compensating drop in the cost of a lower protein diet.

In comparing figure 4, *A* and *B*, it should be kept in mind that 100 percent of the maximum live weight in the limited-fed group is equal to an average of 88 percent of the maximum live weight of the group fed ad libitum.

Since the Spillman ratio, *R*, is equal to e^{-k}, the values of *R* fall in inverse order to the values of *k*. From this it follows that the more rapid the rate of decrease in efficiency the lower the corresponding Spillman ratio. In this study the low protein diets gave the slowest rate of decrease in efficiency because of their low initial efficiencies; also the 25-percent protein diet fed ad libitum had a low rate of decrease in efficiency for the same reason. It is evident that as the efficiency of utilization of the diets increased as a result of the increase in protein content, the magnitude of the Spillman ratios of the diets decreased. This is due to the fact that all lots of birds within each group reached approximately the same mean live weight at maturity.

For those who have followed the work of Spillman (Spillman and Lang (*14*)) and that of Titus, Jull, and Hendricks (*17*), this finding may seem to contradict previous results. When the same diet is fed at different levels of intake, as in the experiment made by Titus and associates (*17*), the initial efficiencies are nearly equal, but the final live weights for the different levels of intake are in the same order as the energy content of the feed consumed. Under such conditions the lots that attained the greatest average live weight would have the slowest rate of decrease in efficiency and hence the highest Spillman ratio. Thus it is possible for the Spillman ratio to be affected in one way when the feed intake is reduced but in the opposite way when the protein content is reduced. This statement is also true of *k* and *C*. Hence, any one of these constants by itself is of little value in evaluating the desirability of a diet. However, when *A*, *k*, and *C*, or *A*, *R*, and *C*, are taken into consideration at the same time they constitute an excellent basis for comparing the desirability of diets.

The utilization of the protein in the diet for growth is shown in a convenient form in figure 3, *A* and *B*. The facts shown by figure 3 are substantiated by the values of

$$\frac{C}{\text{percentage of protein in the diet}}$$

in table 6 and by figure 5, *A* and *B*, showing the straight-line relationship between

$$\frac{E}{\text{percentage of protein in the diet}}$$

and *W*, expressed as percentage of *A*. All evidence points to the same conclusion, namely, that with the exception of the lot fed the 13-percent protein diet, the efficiency of protein utilization by the lots in the ad libitum-fed group decreased with each increase in increment of protein. In the limited-fed group the same was true for the diets containing 17 percent or more of protein. It also follows that, since the more protein the birds ate the faster they grew, the faster the birds grew the less efficiently they used the protein of their diets.

After mature weight had been reached, the birds on the diet that contained only 13 percent of protein maintained their live weight as well as those that received higher percentages of protein.

SUMMARY AND CONCLUSIONS

Comprehensive data were obtained on the relationship between the level of protein intake and live weight, feed utilization, and protein utilization of cross-bred male chickens. Rhode Island Red males and Barred Plymouth Rock females comprised the parent stock. Diets ranging from 13 to 25 percent of crude protein, by increments of 2 percent, were fed to seven pens of males ad libitum and to seven pens of males at 70 percent of an idealized intake for this cross for a period of 52 weeks. The weight of feed consumed per chicken per week and the live weight of the chickens at the end of each week were obtained.

Standard errors of the mean live weights were calculated at several ages, and the statistical significance of the differences between the means determined. The smoothed average live weights were plotted against age, feed consumption, and protein consumption.

The equation of the curve of diminishing increment was fitted to the data recorded for each of the 14 lots of chickens. Tabulations were made of the following parameters and their standard errors: A, the maximum live weight attainable; C, the efficiency of the diet when there is no maintenance requirement; B, the maximum gain attainable; k, the rate of decrease in efficiency; and R, the Spillman ratio.

Values of kB, the efficiency at first feeding, and

$$\frac{C}{\text{percentage of protein in the diet}},$$

the maximum gain theoretically possible per unit of protein consumed, were computed. Likewise the values of

$$\frac{E}{\text{percentage of protein in the diet}}$$

were plotted against W expressed as percentage of A.

When E was plotted against W expressed as percentage of A, it was found that the decline in efficiencies followed straight lines that reached a common zero point at A. Thus, it was shown that the efficiencies of feed utilization on the several diets at any specified live weight were in the same order as the values of C for those diets. In a similar manner the gains per unit of protein consumed on the different diets at a given live weight were shown to fall in the same order as the values of

$$\frac{C}{\text{percentage of protein in the diet}}$$

for those diets.

During the first 14 weeks the average live weights were found to be in the order of the quantity of protein consumed. After the fourteenth week the advantage in growth previously obtained by the birds on the higher protein diets was maintained to maturity but no further advantage was gained.

On the basis of feed consumed the chickens fed 21 percent of protein were the most efficient of the lots fed ad libitum, and those fed 25 percent of protein were the most efficient of the limited-fed lots.

On the basis of protein consumed, the chickens fed 17 percent of protein were the most efficient of the limited-fed lots and those fed 15 percent of protein were the most efficient of the ad libitum-fed lots.

Decrease in the efficiency of feed or protein utilization was found to be a linear function of live weights.

The more closely the birds approached their maximum live weight the smaller became the difference between the efficiencies of feed utilization.

All lots within the group fed ad libitum reached approximately the same live weight by the fortieth week. All lots in the limited-fed group reached approximately equal live weights by the fifty-second week. The more protein a lot of birds consumed the sooner the birds in that lot reached maturity.

The birds in the limited-fed lots used their feed more efficiently up to a live weight of 1,500 g, although they gained more slowly than those in the lots fed ad libitum. The high initial efficiencies of the former lots decreased more rapidly than the relatively low initial efficiencies of the latter lots. As a result, they required considerably more feed to attain an average weight of 2,700 g than did the lots fed the same diets ad libitum.

When the feed consumption was restricted to 70 percent of the normal intake, the Spillman ratio decreased as the level of protein intake increased. The result was the same when the birds were allowed to eat all they wanted, but in this case the decrease continued only until the level of protein intake was 21 percent, after which the Spillman ratio tended to increase.

For birds of the same age the more protein consumed per bird per week the greater is the average gain per week until the inflection point on the growth curve is reached.

Accurate comparison of the efficiencies of feed utilization is greatly simplified if efficiency is considered as a function of live weight rather than as a function of age.

For birds of the same live weight the relative position of the efficiencies of a series of adequate diets fed under identical conditions always tends to be the same.

When the diet contains all the factors necessary for growth, the curve followed by the relative rates of gain is similar in shape to an inverted curve of diminishing increment, and its general type is not changed by the protein content of the diet.

The constants of the curve of diminishing increment should be used in the combinations A, k, and C, or A, R, and C, but never singly, to evaluate a diet.

Of the diets used in this experiment, when consumed ad libitum, the one that contained 21 percent of protein was the most efficient for growth. It is therefore concluded that 21 percent of protein is near the optimum for growing chickens of all ages when the diet is fed ad libitum. In the case of the limited-fed group the efficiency of feed utilization continued to increase as the level of protein intake was increased from 13 to 25 percent.

After a bird has reached approximately half of its mature weight the difference in efficiency between a diet containing 21 percent of

protein and one containing 17 or even 15 percent of protein may be small enough to justify, in the interest of economy, the use of a diet containing the lower percentage of protein.

After maximum live weight has been reached, 13 percent of protein is adequate for the maintenance of male chickens.

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ENERGY REQUIREMENTS OF SWINE AND ESTIMATES OF HEAT PRODUCTION AND GASEOUS EXCHANGE FOR USE IN PLANNING THE VENTILATION OF HOG HOUSES [1]

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INTRODUCTION

A few years ago, the writers (*24*)[2] published estimates of the daily output of heat, carbon dioxide, and water by various kinds and conditions of poultry for the use of the agricultural engineer in planning the ventilation of poultry houses. The present paper is an extension of this line of work to swine, supplementing and, to some extent, confirming the somewhat fragmentary estimates previously made by Armsby and Kriss (*2*).

The data presented in this paper are also of significance to the problem of the energy requirements of growing, gestating, and lactating swine. They are the result of an analytical study of the energy requirements of swine for each of the various animal functions as revealed by published information, and of an integration of these requirements to apply to pigs of any particular age (or weight) and condition. This method of study, involving the differentiation of animal functions for separate measurement and study and the integration of these measurements for application to any particular animal, is scientifically sound and, because of its flexibility, is widely applicable. If at this writing the method cannot be followed through with perfect assurance with reference to any particular species of animal, the difficulties in so doing will be perfectly obvious and will indicate clearly the most significant problems for future research.

METHODS OF ESTIMATION

The general procedure followed in estimating the energy requirements of swine has been to factor out and to assess as accurately as possible all of the items contributing to the expenditure and storage of energy, and then to integrate these factors for pigs of different weights and condition. Estimates of the net availability of the metabolizable energy of food permit the conversion of net energy to metabolizable energy values, and from the latter, dry-matter requirements may be estimated for some more or less typical ration. Summation of energy expenditures (basal metabolism, muscular activity, heat increment of feed) leads to an estimate of heat production, and from the latter estimates of the probable output of carbon dioxide and of the usual and maximal output of water vapor may be made. The sources of information in all these estimations will first be considered.

[1] Received for publication December 11, 1937; issued June 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 827.

Basal metabolism.—The basal metabolism of pigs of different weights has been quite extensively studied by Brody (*7*) and by Deighton (*10*), using castrated male pigs and female pigs. The measurements obtained have been analyzed statistically by Brody. For pigs weighing from 50 to 275 kg, the equations fitted to the two sets of data give predictions that agree very closely. For pigs weighing less than 50 kg., Brody's data are too few to confirm or refute the data of Deighton. In estimating the basal metabolism of castrated male and female pigs for the purposes of this report, the prediction equation derived by Brody from Deighton's data have been used. These equations are—

For pigs weighing less than 50 kg:

$$\frac{Q}{m}=68e^{-0.049m}+22.$$

For pigs weighing more than 50 kg:

$$\frac{Q}{m}=34e^{-0.011m}+10$$

where Q is the basal heat production in calories per day, m is the weight of the pig in kilograms, and e is the base of natural logarithms.

For uncastrated male pigs, the data of Brody appear to be the only information available, the prediction equation being

$$\frac{Q}{m}=34e^{-0.0162m}+17.$$

Available information does not indicate any considerable differences in the basal metabolism of pigs due to breed or type, Deighton's data (*10*) notwithstanding.

Muscular activity.—The maintenance energy requirement of an animal includes, besides the basal expenditure, a quota of energy expended in a degree of muscular activity presumably more or less characteristic of the species. The size of this quota has not been measured with any considerable degree of accuracy either for the pig or for other farm animals, although the information concerning this energy quota and its variation with age, sex, degree of confinement, environmental conditions, etc., would possess much scientific and practical importance. Wood (*36*) has attempted an indirect determination of this quota by deducting from the estimated net energy intake of various groups of growing pigs the energy estimated to have been expended in the basal metabolism and the energy estimated to have been stored in the tissues. While the results vary greatly, they led Wood to the conclusion (*36, p. 439*) that—

> the normal daily expenditure of energy on muscular effort by a well-fed and tended Large White pig is in the neighborhood of 1000 calories per day, which is equivalent to 1 lb. per day of any standard mixed pig meal. This figure varies widely from week to week according as the conditions induce more or less muscular movement. If the animals are quiet and contented it is low. If they have to scramble for room at the trough or if they are distressed by hot weather it is high. As it yields no return at all in the form of increased live weight, the importance of keeping it low by good management is strongly emphasized.

The daily expenditure of energy for muscular activity appeared to be as large for the little pig as for the big one.

While it is difficult to point out any gross error in these calculations of Wood, there is very good reason to believe the estimate of the activity energy quota is considerably too high. Mitchell and Hamilton (*23*) found that 1 pound a day per 100 pounds live weight of a ration composed of corn, middlings, and tankage was adequate to maintain pigs weighing 225 pounds in energy equilibrium under the practical conditions of dry-lot feeding. For pigs weighing 50 to 70 pounds, 1.5 pounds of the ration used was required per 100 pounds live weight. Furthermore, the determined metabolizable energy intake required for maintenance of energy equilibrium (1,015 cal.) exceeded the estimated basal metabolism (841 cal.) by only 174 cal. for 50-pound pigs, and by only 1,295 cal. (3,445−2,150) for 225-pound pigs. Assuming a net availability of the metabolizable energy of 80 percent for maintenance (see below), leaves no quota of energy for activity for the 50-pound pigs and only 600 cal. for the 225-pound pigs.

Breirem (*6*), working in Møllgaard's laboratory, estimates the maintenance requirement of pigs from the equation

$$M=2{,}101\left(\frac{W}{100}\right)^{.56}$$

where M is the daily requirement of net energy (for pigs, not for cattle, as in the original formula; the constant $2{,}101=1{,}450\div 0.69$) and W is the body weight of the pig in kilograms. This formula, obtained from experimental data on bacon-type pigs, gives very nearly the same values for maintenance as Brody's equations (based on Deighton's data) give for basal metabolism (table 1).

TABLE 1.—*The maintenance requirement of energy for pigs by different standards*

Weight of pig (pounds)	Daily basal metabolism (Deighton)	Daily maintenance requirement according to—			Weight of pig (pounds)	Daily basal metabolism (Deighton)	Daily maintenance requirement according to—		
		Breirem	Edin and Helleday				Breirem	Edin and Helleday	
	Calories	*Calories*	*Feed units*	*Calories*		*Calories*	*Calories*	*Feed units*	*Calories*
50	1,007	921	0.53	1,167	150	1,775	1,695	1.09	2,400
75	1,185	1,154	.73	1,607	175	1,921	1,847	1.13	2,488
100	1,332	1,353	.90	1,982	200	2,045	1,989	1.12	2,466
125	1,544	1,532	1.02	2,246	225	2,150	2,124	1.07	2,356

Breirem adds to his maintenance quota of energy, a quota for production in order to obtain a total requirement. Thus, in this scheme, no provision is made for a quota of energy dependent upon muscular activity outside of that contained in the maintenance requirement.

From Swedish cooperative feeding tests, Edin and Helleday (*12*) estimate the maintenance requirements of growing swine in feed units by the following equation:

$$y=2.72+0.0164x$$

in which y=feed units required per 100 kg body weight and x=body weight in kilograms. The feed units so computed are given in table 1. In converting these feed units into net energy, use is made (1) of Popoff's (*26*) recent determination of the net energy value of barley for the

fattening of swine, i. e., 1,960 cal. per kilogram, and (2) of Breirem's finding (*6*) that in swine the net energy of a feed for fattening is only 89 percent of its net energy for maintenance. On these assumptions, 1 kg of barley, or 1 feed unit, would have a net energy value for maintenance of 2,202 cal. The net energy requirements for the malntenance of swine thus computed from Edin and Helleday's equation are given in column 5 of table 1. They show increments over the Deighton basal values ranging from 160 to 702 cal. daily, or, in percent of the basal, from 10 to 49, averaging about 30.

Rapport, Weiss, and Csonka (*27*) noted increases of heat production of 12 percent when a pig was restless in the respiration chamber, and an increase of only 35 percent when "much movement was noted."

Contrary to the conclusion of Wood, it appears that the energy expenditure by swine in muscular activity is a comparatively small item. For the purposes of these estimates, it has been assumed that this quota equals 20 percent of the basal energy expenditure for swine of all ages and conditions. Most certainly the information on this point needs confirmation and supplementation.

Growth and fattening.—The rate of body weight gain by growing and fattening pigs has been taken from Missouri (*15*) and Ohio (*28*) records and represents a liberal system of feeding. According to Hogan and his coworkers (*15*), there is no difference between the bacon and lard types of pigs as regards rate of gain in the feed lot or composition of gain on the same ration, while later Illinois (*9*, *23*) results indicate the same to be true for different types of Poland China pigs, except for a possible inferiority of the Chuffy type with respect to rate of gain. Type differences relate to the distribution of fat on the carcass, not to the fat content of the added tissue.

While type of pig has not been shown to affect energy requirements, the method of feeding employed will determine to some extent not only the rate of growth and fattening, but also the composition of the gains put on. Hence, estimates were made separately for what has been called American feeding practice and English feeding practice. For the latter, the data of Wood (*36*) on rate of gain and the composition of gain have been used. However, Whetham's (*31*) results indicate a more even rate of gain of pigs shown at the Smithfield carcass competitions, averaging 1.22 pounds per day. For the estimates of composition of gain according to American feeding practice, the data of Hogan and his coworkers (*15*) and of Mitchell and Hamilton (*23*) have been considered. The estimates finally used for the energy value of a pound of gain at the various body weights are given in table 2.

TABLE 2.—*Estimated energy content of a pound of gain by pigs of increasing body weight*

Body weight (pounds)	Energy content of gain per pound	Body weight (pounds)	Energy content of gain per pound	Body weight (pounds)	Energy content of gain per pound	Body weight (pounds)	Energy content of gain per pound
	Calories		*Calories*		*Calories*		*Calories*
2.5	500	75	1,625	200	2,400	500	3,950
5	750	100	1,750	225	2,600	600	4,050
10	1,000	125	1,850	250	2,850		
25	1,250	150	2,025	300	3,200		
50	1,500	175	2,200	400	3,750		

Pregnancy.—In estimating the requirements of a pregnant 300-pound gilt and a 400-pound sow, it has been assumed that the former would be fed to gain 1 pound daily and the latter 0.6 pound. It has been further assumed that, except for the increasing weight of the products of conception, the energy content of the gain in body weight would contain 2,400 and 3,000 cal. per pound, respectively. The gains in weight of the products of conception throughout the gestation period of 16 weeks, as well as the energy content of these products, have been taken from the publication of Mitchell, Carroll, Hamilton, and Hunt (*21, table 16*). The estimates relate to an average litter of eight pigs.

Lactation.—In estimating the energy requirements of a lactating 400-pound sow, it is assumed that she would be maintained in weight and energy equilibrium throughout an 8-week lactation period. This ideal would probably be only rarely realized in practice, and to the extent that practice falls short of this ideal, the estimates of probable output of heat, water vapor, and carbon dioxide are too high. The daily yield of milk is estimated from the compilation of data published by Bonsma and Oosthuizen (*3*), while the energy content of sow's milk, 654 cal. per pound, is computed from the analyses of Hughes and Hart (*17*).

Net energy required.—The total requirement of net energy is equal to the sum of all expenditures of energy (basal metabolism, muscular activity) with the exception of the heat increment due to feed, and all storages of energy in growth, fattening, gestation, and lactation.

Metabolizable energy required.—In estimating the requirements of metabolizable energy from the expenditures of energy in the basal metabolism and in muscular activity, and from the storages of energy, it was assumed that the metabolizable energy was 80 percent net available when used for maintenance and milk production, and 70 percent net available when used for growth and fattening and for intrauterine growth.

These values represent compromises between more or less discordant data. From respiration experiments on swine carried out in Møllgaard's laboratory, Breirem (*6*) concludes that the metabolizable energy of the rations fed was 81 percent net available for maintenance. Fingerling (*13*) obtained an average utilization of 75 percent for the metabolizable energy of the basal rations used in his respiration experiments on swine. These rations were all supermaintenance with respect to energy.

The data on the utilization of energy for fattening are much more numerous. From a number of German experiments on swine, Armsby (*1*) has computed the net availability of the metabolizable energy of swine feeds for fattening, the estimates varying from 69 to 85 percent and averaging 79. Fingerling (*13*) found that the metabolizable energy of potato starch added to supermaintenance basal rations was utilized to an average extent of 83.5 percent. Breirem (*6*) obtained a net utilization of only 70 percent of the metabolizable energy intake of swine for growth and fattening. Feeding and slaughter tests on growing swine at the Illinois Agricultural Experiment Station (*23*) indicated an average net availability of 63 percent of the metabolizable energy consumed above maintenance. Similar experiments of Popoff (*26*), in which barley was added to a basal ration permitting some fattening, were interpreted to mean that barley possesses a net energy

value of 97.8 therms per 100 pounds of dry matter. Assuming the metabolizable energy of barley to be 151 therms per 100 pounds of dry matter (*1, table 204*), the net availability of the metabolizable energy amounts to 65 percent. From Wellmann's feeding and slaughter experiments (*30*) on eight suckling pigs, the writers have computed an average net availability of the metabolizable energy of 84.5 percent for both maintenance and growth.

Dry matter required.—In converting the requirements of metabolizable energy into terms of dry matter, the latter has been assumed to contain 3,350 cal. per kilogram; this value refers in particular to a ration of corn, tankage, and middlings (*23*).

Total heat produced.—The total heat produced by a pig of any age or condition is the sum of the heat expended in maintenance (basal metabolism and muscular activity) and the heat increment due to feeding. The latter increment is the difference between the requirement for metabolizable energy and that for net energy.

Output of water vapor.—The output of water vapor is computed for all pigs under two sets of conditions. When the temperatue of the environment is equal to or is above the body temperature of the pig, i. e., 102.5° F., all of the body heat will be lost in the evaporation of water. In this extreme case, the daily loss of water vapor in grams will be equal to the daily heat production in calories divided by 0.58, the heat of vaporization of 1 g of water at approximate skin temperature, 85° F. or so. The percentage of the total heat output lost in the vaporization of water may vary widely from this maximum as environmental conditions vary. It will decrease to zero whenever the relative atmospheric humidity equals 100 percent. At low temperatures it will be small and will increase along a logarithmic curve (*24, p. 737*). It is commonly considered that under usual conditions animals lose about 25 percent of their heat in the vaporization of water, and this has been assumed in the computations given below, particularly since Wierzuchowski and Ling (*32*) as well as Rapport, Weiss, and Csonka (*27*) have shown that pigs in the respiration chamber excrete 20 to 25 percent of their heat in the vaporization of water.

Output of carbon dioxide.—Although extremely high respiratory quotients (1.40 to 1.58) have been noted for pigs during short periods of time in the absorptive period (*32*), the 51 respiration experiments of Breirem (*6*) on pigs fed high- and low-protein rations, indicate a range in respiratory quotients of only 0.99 to 1.25, averaging about 1.1, for 24-hour periods. An average respiratory quotient of 1.1 has been assumed in estimating the carbon dioxide production of pigs from their production of heat. At this respiratory quotient 1 liter of oxygen possesses a calorific value of 5.156 cal. It is evident that the output of carbon dioxide indicated in the tables may at times be exceeded by 40 to 50 percent.

Estimation of critical temperature.—In estimating the critical temperature of pigs of any particular weight and condition, that is, the temperature below which extra heat must be generated to maintain body temperature, the critical temperature of the fasting pig is taken at 68° F., on the basis of the experiments of Capstick and Wood (*8*). The rise in heat production below the critical temperature seems to proceed along a straight line (*8*) and to follow Newton's law of cooling bodies (*22, p. 556*). Hence the extra heat required per degree Fah-

renheit drop in environmental temperature to maintain the body temperature of the pig is given in each case by the equation

$$k=\frac{BM}{34}$$

where BM is the basal heat production per day, and 34 is the difference between the body temperature of the pig, 102° F., and the assumed critical temperature under basal conditions, 68°. The heat available to meet this added demand is provided by muscular activity and by the heat increment of feeding. Hence, the sum of these two sources of heat, divided by k as above determined, gives the range in environmental temperature below the critical temperature of fasting within which the available supply of heat is adequate to maintain body temperature. The lower limit of this range is therefore the critical one under the specified conditions of activity and feeding.

It is of course true that calculations of this character are not particularly reliable, because the critical temperature of an animal will be lowered by any condition that increases heat production (activity, feeding) or insulates the animal from its environment (increasing fatness, hair growth); it will be raised by any condition that decreases heat production (sleep) or increases heat emission at low temperatures (drafts, increasing humidity). Deighton (*10*) has obtained indications of a much lower critical temperature (16° C.) in a Middle White pig than that more accurately measured by Capstick and Wood (*8*). Since the pig used in the latter study varied in weight from about 200 to 400 pounds during the period of observation, the calculated critical temperatures for pigs within this weight range may be more significant than those calculated for lighter or heavier pigs.

Estimates for boar pigs.—The data reported in the tables of this paper relate to female pigs and castrated male pigs, the assumption being that females and unsexed males are similar with reference to the physiological measurements considered (*23*). No data on boar pigs with reference to muscular activity and composition of gains were found in the literature. One comparison of the growth rates of male and female pigs was discovered (*33*), indicating a much more rapid growth for the former. Brody has demonstrated increases in basal metabolism of 10 to 38 percent in male pigs over females of the same body weight, the percentage sex increment increasing with increasing weight. Popoff's experiments (*26*) on the net energy of barley was carried out on boar pigs. However, these bits of information are inadequate to permit estimations for boar pigs of the energy requirements and the heat and gaseous exchange.

Undoubtedly the full male pig is distinguished from the female pig of equal weight by a more intense basal metabolism, a greater rate of growth, and a lower energy content of gains; possibly by a greater degree of muscular activity. Thus, the rate of growth of castrated male pigs may be greatly increased by administering by mouth a testicular preparation (*20*, *34*). The requirements of the boar for energy are probably greater, as would be its production of heat, carbon dioxide, and water vapor.

THE RESULTS OBTAINED

The results of the estimations above described are collected in tables 3, 5, and 6.

TABLE 3.—*Daily food requirements, heat production, and gaseous exchange of growing and fattening female and castrated male pigs, according to American and English feeding practices*

AMERICAN PRACTICE

Body weights (pounds)	Average daily gain	Total net energy required	Metabolizable energy required	Dry matter required	Feed[1] economy of gains	Total heat produced	Water vaporized		Carbon dioxide produced	Estimation of critical temperature		
							Under ordinary conditions	At 103° F. and above		Extra heat available	Heat required per degree drop below 68.7° F.	Critical temperature
	Pounds	*Calories*	*Calories*	*Grams*	*Pounds*	*Calories*	*Grams*	*Grams*	*Liters*	*Calories*	*Calories*	° *F.*
2.5	0.20	250	330	99	1.2	200	86	345	43	100	------	------
5	.30	450	610	182	1.5	380	164	655	81	200	------	------
10	.40	820	1,100	328	2.0	800	483	1,379	171	350	------	------
25	.60	1,575	2,110	630	2.6	1,350	582	2,328	288	670	20	35
50	.85	2,400	3,200	955	2.8	1,980	854	3,414	422	1,010	29	34
75	1.02	3,200	4,500	1,343	3.3	2,550	1,099	4,397	544	1,350	33	28
100	1.20	4,000	5,600	1,672	3.5	3,050	1,315	5,259	651	1,700	39	25
125	1.40	4,700	6,600	1,970	3.5	3,550	1,530	6,121	757	2,000	45	24
150	1.55	5,500	7,600	2,269	3.7	4,000	1,724	6,897	853	2,320	51	23
175	1.70	6,200	8,500	2,537	3.7	4,450	1,918	7,672	949	2,650	55	21
200	1.78	6,900	9,500	2,836	4.0	4,940	2,129	8,517	1,054	2,940	58	18
225	1.85	7,600	10,500	3,134	4.2	5,350	2,306	9,224	1,141	3,210	61	16
250	1.85	8,300	11,300	3,373	4.6	5,700	2,457	9,828	1,216	3,500	62	13
300	1.80	9,250	12,700	3,791	5.3	6,200	2,673	10,690	1,323	3,900	68	12
400	1.60	9,200	12,500	3,731	5.8	6,500	2,802	11,207	1,387	3,850	76	18
500	1.33	8,675	11,650	3,478	6.5	6,600	2,845	11,379	1,408	3,650	86	26
600	1.00	8,000	10,700	3,194	8.0	6,700	2,888	11,552	1,429	3,350	96	30

ENGLISH PRACTICE

2.5	0.15	268	359	107	1.8	209	90	360	45	111	------	------
5	.23	444	593	177	1.9	375	162	647	80	187	------	------
10	.37	719	954	285	1.9	651	280	1,122	139	304	------	------
25	.65	1,285	1,688	504	1.9	1,233	531	2,126	263	541	20	42
50	.97	1,887	2,462	735	1.9	1,783	768	3,074	380	776	29	42
75	1.25	2,422	3,207	957	1.9	2,207	951	3,805	471	1,022	34	39
100	1.50	3,098	4,141	1,236	2.1	2,641	1,138	4,553	563	1,309	39	35
125	1.73	3,929	5,282	1,577	2.3	3,206	1,382	5,528	684	1,662	45	32
150	1.95	5,035	6,813	2,034	2.6	3,908	1,684	6,738	834	2,133	52	27
175	2.03	5,756	7,812	2,332	2.9	4,361	1,880	7,519	930	2,440	56	25
200	1.95	6,354	8,639	2,579	3.3	4,739	2,043	8,171	1,011	2,694	59	23
225	1.75	6,955	9,475	2,828	4.0	5,100	2,198	8,793	1,088	2,950	62	21
250	1.55	8,115	11,113	3,317	5.4	5,688	2,452	9,807	1,214	3,446	65	16

[1] Expressed in pounds of food (containing 12 percent of moisture) required per pound of gain.

An important test of these more or less uncertain estimates of the nutritive requirements of pigs is to compare them with estimates and feeding standards proposed on the basis of other evidence. Also, for the growing and fattening pigs, the feed economy of gains has been computed in the ordinary way, after converting the dry matter requirements into requirements for feed containing 12 percent of moisture. These ratios of feed to gain are given in table 3. For

the feeding according to American practice, the ratios are those ordinarily obtained, but for the feeding according to English practice, the ratios seem low, the fattening process being much slower.

TABLE 4.—*Comparions of estimated feed requirements of growing and fattening pigs with various proposed standards*

Body weight (pounds)	Estimated feed requirements [1]		Proposed feeding standards of others for comparison			
	American practice	English practice	Brierem [2]	Edin and Helleday [2]	Hansson and Bengtsson [2]	Morrison [1] (minimum values)
	Kilograms	*Kilograms*	*Feed units*	*Feed units*	*Feed units*	*Kilograms*
50	1.1	0.8	0.9	1.2	1.2	1.1
75	1.5	1.1	1.3	1.7	1.7	1.5
100	1.9	1.4	1.6	2.2	2.1	1.9
125	2.2	1.8	1.9	2.5	2.5	----
150	2.6	2.3	2.2	2.8	2.8	2.5
175	2.9	2.6	2.5	3.1	3.0	----
200	3.2	2.9	2.8	3.3	3.2	3.0
225	3.6	3.2	3.1	3.5	----	----
250	3.8	3.8	----	----	----	3.4
300	4.3	----	----	----	----	3.7

[1] Expressed in terms of feed containing 12 percent of moisture.
[2] Expressed in feed units of 1 kg of concentrate feed mixture.

In table 4 the estimated requirements (in kilograms) of growing and fattening pigs for feed containing 12 percent of moisture are compared with the recommendations of Breirem (*6*), Edin and Helleday (*12*), and Hansson and Bengtsson (*14*) expressed in Scandinavian feed units, and with the lower values in the feeding standard of Morrison (*25*), expressed in kilograms of feed containing 12 percent of moisture. All the values given are mutually comparable.

The agreement between the proposed estimates and those made by other investigators is in general remarkably good. The recommendations of Breirem check best the estimates for the English system of feeding, while the other recommendations check best with those for the American system, this being true in particular for the Morrison standard up to 200 pounds body weight.

From Breirem's (*5*) feeding experiments with breeding sows, it may be calculated that a 300-pound gilt would require 1.1 feed units daily for maintenance and 0.5 feed unit additional for the requirements of pregnancy. This may be compared with the average estimate of about 1.8 kg of dry matter daily (table 5). In the case of a 400-pound sow, Breirem's estimate would call for 1.85 feed units daily, while the estimates of table 5 range from 1.4 to 1.9 kg of dry matter daily. For a 300-pound gilt Morrison (*25*) recommends 2.0 kg of dry matter daily, and for a 400-pound sow, 2.1 kg.

For lactating sows, Breirem (*4*) recommends 0.5 to 0.6 feed unit per pig per day above the needs of the sow. In the case of a 400-pound sow suckling eight pigs, the average daily requirement would be 5.75 feed units, a figure greatly in excess of the dry-matter requirements estimated in table 6, which range from 2.4 to 3.2 kg daily, equivalent to 2.7 to 3.6 kg of feed containing 12 percent of moisture. Morrison recommends a minimum of 4.3 kg of dry matter per day for a 400-pound sow nursing a litter.

TABLE 5.—*Daily food requirements, heat production, and gaseous exchange of a pregnant 300-pound gilt throughout gestation, fed to gain 1.0 pound daily, and of a pregnant 400-pound sow throughout gestation, fed to gain 0.6 pound daily*

At end of gestation week—	Body weight	Total net energy required	Metabolizable energy required	Dry matter required	Total heat produced	Water vaporized		Carbon dioxide produced
						Under ordinary conditions	At 103° F. and above	
	Pounds	*Calories*	*Calories*	*Grams*	*Calories*	*Grams*	*Grams*	*Liters*
300-POUND GILT [1]								
0	300	5,380	7,171	2,141	4,671	2,013	8,053	996
1	307	5,257	6,992	2,088	4,637	1,999	7,995	989
2	314	5,159	6,848	2,044	4,612	1,988	7,952	984
3	321	5,068	6,714	2,004	4,591	1,979	7,916	979
4	328	4,987	6,595	1,969	4,574	1,971	7,886	976
5	335	4,910	6,481	1,935	4,559	1,965	7,860	973
6	342	4,832	6,366	1,900	4,544	1,958	7,834	969
7	349	4,765	6,266	1,870	4,532	1,953	7,814	967
8	356	4,699	6,167	1,841	4,521	1,949	7,795	964
9	363	4,638	6,077	1,814	4,513	1,945	7,781	963
10	370	4,581	5,991	1,788	4,506	1,942	7,769	961
11	377	4,522	5,904	1,762	4,500	1,940	7,759	960
12	384	4,466	5,820	1,737	4,493	1,937	7,747	959
13	391	4,422	5,752	1,717	4,491	1,936	7,743	958
14	398	4,370	5,675	1,694	4,487	1,934	7,736	957
15	405	4,326	5,668	1,674	4,486	1,933	7,734	957
16	412	4,280	5,539	1,653	4,485	1,933	7,773	957
400-POUND SOW [2]								
0	400	4,950	6,509	1,943	4,709	2,030	8,119	1,005
1	404	4,788	6,275	1,873	4,653	2,005	8,022	993
2	408	4,660	6,089	1,818	4,610	1,987	7,948	984
3	413	4,539	5,913	1,765	4,571	1,970	7,881	975
4	417	4,432	5,758	1,719	4,540	1,957	7,828	969
5	421	4,326	5,603	1,673	4,506	1,942	7,769	961
6	425	4,223	5,453	1,628	4,475	1,929	7,716	955
7	429	4,126	5,312	1,586	4,446	1,917	7,666	949
8	434	4,034	5,177	1,545	4,420	1,905	7,621	943
9	438	3,948	5,052	1,508	4,397	1,895	7,581	938
10	442	3,867	4,934	1,473	4,375	1,886	7,543	933
11	446	3,779	4,805	1,434	4,350	1,875	7,500	928
12	450	3,699	4,687	1,399	4,329	1,866	7,464	924
13	455	3,628	4,584	1,368	4,312	1,858	7,434	920
14	459	3,585	4,520	1,349	4,307	1,856	7,426	919
15	463	3,630	4,581	1,367	4,339	1,870	7,481	926
16	468	3,675	4,643	1,386	4,371	1,884	7,536	933

[1] Critical temperature at 0 week=36° F.; at 16 weeks = 46°.
[2] Critical temperature at 0 week = 41° F.; at 16 weeks = 50°.

TABLE 6.—*Daily food requirements, heat production, and gaseous exchange of a lactating 400-pound sow, fed to maintain body weight* [1]

Week of lactation	Milk produced daily	Total net energy required	Metabolizable energy required	Dry matter required	Total heat produced	Water vaporized		Carbon dioxide produced
						Under ordinary conditions	At 103° F. and above	
	Pounds	*Calories*	*Calories*	*Grams*	*Calories*	*Grams*	*Grams*	*Liters*
0	0.0	3,150	3,937	1,172	3,937	1,697	6,788	840
First	6.57	7,447	9,309	2,779	5,012	2,160	8,641	1,069
Second	7.86	8,290	10,363	3,093	5,223	2,251	9,005	1,114
Third	8.29	8,572	10,715	3,199	5,293	2,281	9,126	1,129
Fourth	8.00	8,382	10,477	3,127	5,245	2,261	9,043	1,119
Fifth	7.43	8,009	10,011	2,988	5,152	2,221	8,883	1,099
Sixth	6.57	7,447	9,309	2,779	5,012	2,160	8,641	1,069
Seventh	5.86	6,982	8,728	2,605	4,896	2,110	8,441	1,045
Eighth	5.14	6,512	8,140	2,430	4,778	2,059	8,238	1,019

[1] Critical temperature at 0 week=51° F.; at 1 week=37°; at 3 weeks=34°; at 8 weeks=41°.

Wöhlbier (*35*) also estimates much higher energy requirements for a lactating sow than those given in table 6. By interpolating in his table, a 400-pound sow would require during the first 4 weeks of lactation 4.8, 5.1, 5.0, and 6.6 kg starch equivalent, or, taking 1 feed unit to equal 0.7 kg starch equivalent (*12*), 6.8, 7.3, 7.1, and 9.4 feed units. However, these computations are based upon much larger milk yields of 11.9, 15.4, 15.0, and 18.0 pounds daily in the first 4 weeks of lactation. Wöhlbier claims in an earlier article (referred to in (*35*)) that ordinary methods of computing the milk yield of a sow by weighing the litter before and after suckling at regular intervals during a control day may greatly underestimate the milk that the litter would get when given free access to the udder, because the teats of the sow are quickly filled with milk and are also quickly emptied by the suckling pig, in as short a time as 25 to 35 seconds.

If Wöhlbier's contentions are correct, the calculations should be revised in accordance with his larger milk yields. Such a revision will be found in table 7 covering the first 4 weeks of lactation.

TABLE 7.—*Revision of table 6 to conform to Wöhlbier's larger estimates of milk yield*

Week of lactation	Milk produced daily	Total net energy required	Metabolizable energy required	Dry matter required daily	Total heat produced	Water vaporized		Carbon dioxide produced	Critical temperature
						Under ordinary conditions	At 103° F. and above		
	Pounds	*Calories*	*Calories*	*Grams*	*Calories*	*Grams*	*Grams*	*Liters*	*°F.*
First	11.9	10,932	13,665	4,079	5,883	2,536	10,143	1,255	26
Second	15.4	13,222	16,528	4,934	6,456	2,783	11,131	1,377	18
Third	15.0	12,960	16,200	4,836	6,390	2,754	11,017	1,363	18
Fourth	18.0	14,922	18,653	5,568	6,881	2,966	11,864	1,468	13

This revision raises the estimates of dry matter requirements to the estimate of Morrison and even above for the peak of lactation. Expressed on the basis of 12 percent of moisture to make them comparable with Scandinavian feed units, the requirements for successive weeks are 4.6, 5.6, 5.5, and 6.3 kg. Even these are considerably below Wöhlbier's values. This discrepancy must be due to a much lower assumed utilization of metabolizable energy by Wöhlbier.

USE OF BASIC DATA FOR THE DESIGN OF HOG-HOUSE VENTILATION SYSTEM

HOG HOUSE TEMPERATURES

The average rectal temperature of hogs is 102.5 F. with a range from 101.6° to 103.6°. Physiologists (*11*, *29*) tell us that a hog is not a completely thermoregulated animal and at temperatures above 79° physical regulation of body temperature is no longer sufficient. Every hog raiser knows the danger of high summer temperatures and the need of shade. The critical temperature of the hog is shown to be approximately 68° under basal conditions and varies with the plane of nutrition, being lower for large hogs on normal feed (table 3). Capstick and Wood (*8*) found that for each degree Fahrenheit drop below the critical temperature there was an increase in the rate of

metabolism of a little less than 3 percent. At temperatures of 18° pigs have been observed to shiver, and the increased heat production calls for more feed. Thus physiological reactions at the lower temperature limits, coupled with practical observations, suggest the desirability of temperatures not lower than 50° to 55° for a farrowing house. Somewhat lower temperatures are permissible for fattening hogs because of their higher plane of nutrition, but house temperatures approaching freezing should be avoided for all classes. There is need for greatly improving the ordinary farm hog house. Such houses are commonly found with little or no ventilation and are poorly lighted. Where a considerable number of hogs are to be provided for, it is believed that a good hog house, well ventilated and lighted, will show a return, for the capital invested, at least equal to that for any other structure on the farm.

Iowa studies (*16*) reveal that, with the poorer type of hog house, each drop of 10° F. below normal weather temperatures at farrowing time was associated with losses of about 5 percent of the pigs, and that careful housing reduced ordinary losses materially.

It is said that when small pigs are kept too warm they are less thrifty. On the other hand, chills together with dampness are often fatal to small pigs.

DATA FOR THE DESIGN OF HOG HOUSES

A knowledge of the rates of heat production and carbon dioxide and water vapor output of various farm animals as affected by weight, age, feed, and environmental conditions, is essential to the agricultural engineer, as a basis for the scientific design of structures for housing the animals, since the animal heat is utilized not only for maintenance of desirable stable temperatures but also for the removal, by ventilation, of the products of respiration, particularly water vapor. Removal of the water vapor at a rate that will maintain desirable conditions of atmospheric humidity is a distinct aid to maintenance of health and to sanitation.

The data presented in previous pages of this paper furnish an approximate basis for scientific design, though the estimates of water vaporized under ordinary conditions, equivalent to 25 percent of the total heat output, may be in excess of actual vaporization at temperature ranges between 32° and 50° F. These data have been, in part, converted to British thermal units and are shown graphically in figures 1 and 2 in order to bring out the relations of the several factors to each other and to make them more immediately useful to engineers and architects in the design of buildings adapted to maintain adequate ventilation and proper temperatures and thus aid in improving sanitary conditions and decreasing annual losses of early pigs in the community type of hog house.

The data for figure 1 were obtained from table 3 and those for figure 2 from tables 3, 5, and 6.[3] It is recognized that there is no standard relation between age and weight and rate of daily gain, since these vary with feeding practices. However, the plotting of these factors in figure 1 helps one to visualize their approximate relationship under good American feeding practices. Figure 1, *A*, is plotted to an irregular scale of ordinates, so that the normal average

[3] The heat production curve for the 400-pound nursing sow represents minimal estimates. The larger estimates given in table 7 may be more in conformance with practice.

live weight of a pig of any age that has gained according to the rates of gain indicated is shown approximately by reading horizontally across to figure 1, *B*. For convenience the carbon dioxide production

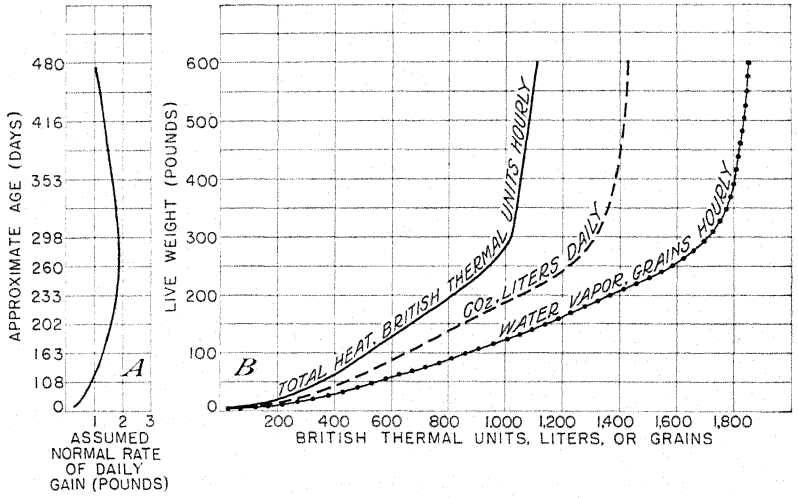


FIGURE 1.—*A*, Approximate normal growth of swine; *B*, total hourly heat and water-vapor production and daily carbon dioxide production for growing and fattening hogs.

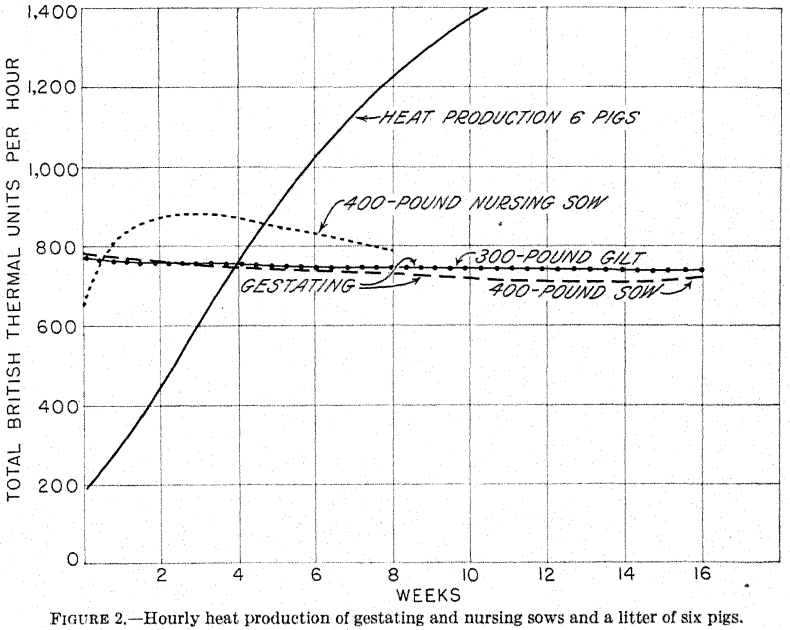


FIGURE 2.—Hourly heat production of gestating and nursing sows and a litter of six pigs.

data in figure 1, *B* are retained in the original form for the present and transposed in an example later. It is noted (fig. 1, *A*) that the rate of gain assumed in these calculations begins to decrease at approximately 250 pounds live weight and that the rate of heat

production as well as of carbon dioxide and water vapor (fig. 1, *B*) increases less rapidly at weights above 300 pounds.

Figure 2 compares the heat production of a 400-pound sow, nursing six pigs, with those of a 300- and a 400-pound sow during the period of gestation. Assuming the rate of gain shown in table 3, the additional heat produced by growing pigs is also shown. It is readily noted that the metabolism of the sow is at a low point after farrowing, but by the end of the first week is above that of the gestating sow, although lower than that of the fattening hog.

It is also noted that the difference in heat production between the 300- and 400-pound sows during gestation is small. The heat production of a male is higher than that of a female. For all practical purposes in making allowance for a boar we may increase the estimate of heat production over that of a sow of the same weight by 10 percent, although in some cases (p. 817) higher allowances may be advisable. These data permit a design for a particular condition and show the possible range of efficient operation of the ventilation system.

VENTILATION REQUIREMENTS [4]

The ventilation requirement of a given animal house may be based upon the heat and moisture production. It would appear that the amount of ventilation that would make efficient use of the heat produced and maintain a dry house would be the most desirable. In order to test out the usefulness of the data presented they are compared briefly with a test, the details of which are recorded elsewhere (*18*). The important features of this test are as follows: It was possible in a good hog house in Minnesota housing 1 boar, 54 fat hogs, and 50 shoats, to maintain fairly uniform temperature and adequate ventilation. Under an outside temperature of 24° F. ranging from 17° to 32°, an average inside temperature of 44° was obtained, ranging from 40° to 50°. The relative humidity ranged from 73 to 84 with an average of 80 percent. The average ventilation maintained was 1,649 cubic feet of air per minute.

In the test house were 26,775 pounds total weight of hogs, which would represent 89 hogs of 300 pounds average weight. However, since rate of heat production varies in young and older hogs, different heat values are required in estimating the heat production of different classes of hogs. By using the values given in figure 1, a total of 81,702 B. t. u. per hour is obtained in the above test house, or an equivalent of 80 head of 300-pound hogs. The average heat production for a 300-pound hog is thus estimated at 1,025 B. t. u. per hour (fig. 1), which is somewhat higher (660 B. t. u.) than that given by Armsby and Kriss (*2*).

If the air in a ventilated hog house is at 50° F. and 80-percent relative humidity, a pound of dry air passing out is accompanied by 43.1 grains of moisture. A pound of dry air entering from outside has a moisture content of 22.5 grains, when the outside air is at 32° and 85-percent relative humidity. Each pound of dry air circulated through the house by ventilation, therefore removes from the house the difference, or $43.1-22.5=20.6$ grains of moisture. Figure 1 shows that a 300-pound hog respires 1,719 grains hourly. To re-

[4] Acknowledgment is made of the assistance and the constructive criticism offered by S. J. Dennis, associate refrigeration engineer, with respect to analysis of heat balance.

move this moisture as fast as it is respired requires an air circulation rate of 1,719÷20.6, or 83.5 pounds of air (dry air) per hour. The specific volumes of the incoming and outgoing air under the given conditions are 12.46 and 12.97 cubic feet per pound of dry air respectively, and the total volume of the incoming air will be 83.5×12.46 or 1,040 cubic feet per hour, and that of the outgoing air 83.5×12.97 or 1,083 cubic feet per hour. This is the ventilation rate required per 300-pound hog to remove the respired moisture as fast as the hog exhales it, assuming that the rate at which water vapor is given off by the hogs is the same at 50° as at 68°. If the rate at which water vapor is given off is less at lower temperatures the ventilation requirements and attendant heat loss will be correspondingly reduced. At present no data for amount of water transpired at low temperatures are available.

The total heat content, under conditions already stated, of the incoming air is 11.17 and that of the outgoing air 18.57 B. t. u. per pound of dry air. Each pound of dry air circulated, therefore, removes a quantity of heat equal to the difference between 18.57 and 11.17=7.40 B. t. u., and the total heat carried out by ventilation at the rate calculated above will be 83.5×7.40=618 B. t. u. per hour, per 300-pound hog. The heat production of a 300-pound hog is 1,025 B. t. u. per hour (fig. 1, *B*). Heat lost by ventilation sufficient to remove the moisture respired by the hog is 618÷1,025×100, or 60.3 percent of the heat produced by the hog. Under the conditions stated above the balance is sufficient to maintain the temperature of an ordinarily well constructed house at the 50° level.

The air volumes required in ventilation sufficient to remove the moisture produced by the hogs, and the heat lost by such ventilation, may be similarly calculated for any given set of temperature and humidity conditions. Data on moisture, heat content, and volume are readily determined by reference to a psychrometric chart or table such as is found in several handbooks on ventilation or air conditioning.

Calculations for various outside and inside temperature conditions will show that when temperatures are low, both inside and outside, ventilation at the rate calculated to remove moisture as fast as given off (assuming a constant rate of production) would involve such large heat losses that all the heat produced by the hog would be carried off by ventilation, leaving no heat available to maintain the temperature of the building. This means that, for practical operation, it will be necessary in cold weather to reduce the amount of ventilation below the calculated requirement, in order to maintain satisfactory temperatures in the hog house. It is obvious that the better the construction and insulation of the house, the less its ventilation will have to be reduced.

Figure 2 shows that the period of low heat production is at farrowing time and during the first week thereafter. Hence for early pigs for zone 1 (*19*) and for the northern part of zone 2 it is often profitable to provide artificial heat during this period. Lanterns, radiant heaters, or furnaces may be used. The cost of installation may range from 85 cents to $6,000 or more for a complete system. However, it is seldom necessary to provide heat for more than a few pens and that but for a few days following farrowing. Scattered dates of farrowing for sev-

eral sows of each herd will decrease the number of heated pens needed. Thus, some form of localized heat or a pig brooder such as the one recently introduced by J. R. Tavernetti and E. N. Hughes, of the California Agricultural Experiment Station, may be had at a small annual cost even if rate of fuel cost is high for a short period. These electric brooders may be made portable and permit of transfer from one pen to the other at the critical period. They provide warmth and protection while the pigs are resting, and the surrounding cooler air stimulates appetite and vigor while the pigs are active. Thus in choosing a form of heat or the temperatures to be maintained in a farrowing house, the farmer must determine the number of sows likely to farrow at one time, the expected outside temperatures, and the number of days that heat will be needed.

SIZE OF FLUE

The total size of the flue in the above hog-house test was 12.2 square feet, and it carried out an average of 1,649 cubic feet of air per minute, with an average velocity of about 135 feet per minute. This volume equals 1,237 cubic feet per hour and the flue size equals 22 square inches per 300-pound hog equivalent. Using the volume of 1,083 cubic feet of air required per hour per head to satisfy ventilation conditions, and the above average flue velocity of 135 feet per minute obtained under actual working conditions, and substituting these values in the usual formula, $Q = V. A.$, it is found that 20 square inches of flue area per 300-pound hog would have been sufficient.

The data shown and methods outlined in this paper should enable the engineer to proceed with increased confidence in the design of structures to meet requirements imposed by various conditions such as capacity of building, class of hogs, and climate.

SUMMARY

An analytical study has been made of the energy requirements of swine for each of the various animal functions, as revealed by published information, and of an integration of these requirements for application to pigs of any particular age, weight, or condition. In this study the various animal functions have been differentiated for separate analysis, the ultimate values established then being applied to any particular animal as needed.

The general procedure has been to estimate the energy requirements of swine by factoring out and assessing as accurately as possible all items contributing to energy expenditure or storage. These factors have then been integrated for pigs of particular weights and conditions. Estimates of the net availability of the metabolizable energy of feed permit the conversion of net energy into metabolizable energy values. From the latter dry-matter requirements have been estimated for definite, more or less typical rations.

Summation of energy expenditures, via basal metabolism, muscular activity and heat increment of feed, leads to an estimate of heat production. From this, estimates of the probable output of carbon dioxide and of the usual and maximal output of water vapor have been made. Complete estimates of this kind have been made for growing and fattening gilts and barrows, both according to American and English feeding

practice, a pregnant gilt and a pregnant sow, and a lactating sow. Tentative estimates of critical temperatures have been made in each case.

The data given and the methods outlined will, it is believed, enable engineers to proceed with increased confidence in the design of structures to meet the requirements imposed by various conditions such as capacity of building, class of hogs, and climate. The findings are transposed into the form of basic data suitable to aid in the design of hog-house ventilation systems and illustrations of thier application are given.

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TOXICITY OF SELENIUM FED TO SWINE IN THE FORM OF SODIUM SELENITE [1]

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INTRODUCTION

Considerable loss of livestock in the north-central Great Plains has been attributed to a disease, known locally as alkali disease, which was considered to be due to the deposits of alkali in the soil. The symptoms manifested by affected animals included loss of hair from the mane and tail of horses, the switch of cattle, and hair from the body of swine. Marked alteration in the growth of the horn of the hoof of all these species was also a prominent symptom, resulting in some cases in a sloughing of the hoof.

In a previous paper (*9*) [3] it was shown that hogs fed at the Bureau of Animal Industry Experiment Station, Bethesda, Md.,[4] on two lots of corn grown in an area where the so-called alkali disease existed developed typical clinical symptoms of this disease. It was found that the two lots of corn contained selenium in the proportion of 10 and 5 parts per million, respectively.

Since this and other information (*1*, *2*, *4*, *5*, *6*, *7*) indicated that selenium in the feed was a responsible factor in the production of the so-called alkali disease, experiments were undertaken to study the effects, on swine, of selenium when given in various quantities mixed with the daily ration.

As selenium in the organic form was not available, these tests were conducted with selenium in the form of an inorganic salt, sodium selenite.

EXPERIMENTAL PROCEDURE

In order to test the effect of feeding selenium in the daily ration, 10 pigs (9 Duroc-Jerseys and 1 Berkshire) about 4 months old were placed in a concrete barn entirely separate from any other pigs at the Bureau's Experiment Station at Bethesda, Md., on April 2, 1934. Each animal had an individual pen about 10 feet long and 5 feet wide, well bedded with straw. Wooden troughs were used for feed and water. Water was kept before the pigs all the time and was from the regular station supply. Each animal was weighed as soon as it was placed on experiment and on several other occasions during the first week to determine the gains made on the normal feed. Weights were taken at weekly intervals thereafter.

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[2] The writers are indebted to H. C. Dudley, formerly of the Bureau of Chemistry and Soils, for the chemical analysis of body parts and fluids for selenium, and to H. G. Byers, of that Bureau, for assistance in preparing the dosage of selenium. Acknowledgment is also made to G. T. Creech, of the Bureau of Animal Industry, for the histopathological examination of the various organs, and to M. S. Shahan, W. B. Shook, C. D. Stein, and C. N. Dale, of that Bureau, for the post-mortem examination of these animals.

[3] Reference is made by number (italic) to Literature Cited, p. 842.

[4] This station, now known as the Animal Disease Station, was moved to Beltsville, Md., in 1935.

The grain feed, which was the mixture fed regularly to hogs at the Experiment Station, consisted of bran 2 parts, corn feed-meal 1 part, alfalfa meal ½ part, and gluten meal 1 part. It was given at the rate of about one-half pound per 10 pounds of body weight a day, and the quantity increased as fast as the animals would consume it. That the feed was adequate is demonstrated by the weekly gains in weight made by the two control pigs (Nos. 4326 and 4327), as shown in table 1. At the end of the first week, selenium in varying quantities was added to the feed of eight pigs (principals), which were divided into pairs. The selenium was given in the form of a tenth molar solution of sodium selenite (17.3 g of dry salt per liter of distilled water). The solution was measured by pipette to give the desired number of parts per million of elementary selenium and was thoroughly mixed with the grain feed, which had been previously weighed and moistened with a measured volume of water.

TABLE 1.—*Weekly quantities of grain feed and selenium consumed and changes in weights of pigs used in experiment*

Pig No.	Week No.	Dry feed		Selenium eaten	Change in weight of pig [1]	Pig No.	Week No.	Dry feed		Selenium eaten	Change in weight of pig [1]
		Given	Eaten					Given	Eaten		
		Ounces	*Ounces*	*Milligrams*	*Pounds*			*Ounces*	*Ounces*	*Milligrams*	*Pounds*
4326 (control)	1	175	175.0	----	+3.5	4332	1	238	238.0	324.0	−1.5
	2	189	189.0	----	+3.5		2	204	64.4	84.0	−.5
	3	210	210.0	----	+3.25		3	238	59.5	77.1	−2.5
	4	238	238.0	----	+2.25		4	238	36.6	48.6	−3.0
	5	266	266.0	----	+5.0		5	204	9.5	12.4	−2.5
	6	294	294.0	----	+5.25		6	238	35.0	45.4	−3.0
	7	329	329.0	----	+3.5		7	272	36.0	47.0	−6.0
	8	350	350.0	----	+5.25		8	204	8.8	11.6	−2.5
	9	378	378.0	----	+5.0		9	238	11.3	14.8	−4.0
4327 (control)	1	154	154.0	----	+2.5	4333	1	175	125.4	171.4	+.5
	2	168	168.0	----	+3.5		2	175	86.4	118.2	+.5
	3	189	189.0	----	+6.0		3	175	56.5	77.2	−1.75
	4	210	210.0	----	−1.5		4	175	20.0	27.2	−4.0
	5	238	238.0	----	+5.5		5	150	7.4	10.1	−.75
	6	252	252.0	----	+2.0		6	175	13.2	18.0	−2.25
	7	280	280.0	----	+5.25		7	200	17.6	24.0	−1.5
	8	308	308.0	----	+4.75		8	150	1.0	1.5	−.5
	9	336	336.0	----	+6.0		9	200	4.9	6.7	−2.0
4328	1	154	24.8	244.8	−4.5		10	150	10.7	14.6	−1.5
	2	154	6.2	60.0	−.5		11	175	19.0	26.0	−2.0
	3	154	8.1	79.2	−3.25		12, 13, 14	550	62.3	85.1	−3.0
	4	154	28.0	288.0	−2.0	4334	1	168	168.0	117.6	+2.5
	5	132	[2] 28.8	[2] 288.0	−.75		2	175	175.0	123.2	+3.0
	6	154	9.4	91.2	−2.0		3	196	[2] 62.0	[2] 43.0	−3.75
	[3] 7	88	8.8	86.4	----		4	196	35.8	24.8	−4.25
4329	1	168	23.5	261.6	−4.25		5	168	31.8	22.0	−.5
	[4] 2	----	----	----	----		6	196	16.2	11.3	−.5
4330	1	182	[2] 43.3	[2] 225.6	−2.25		7	224	32.6	22.5	−1.5
	2	182	[2] 17.9	[2] 93.6	−2.25		8	168	22.6	15.7	−1.0
	3	182	15.3	79.2	−2.75		9	224	26.7	18.5	−2.0
	4	182	14.3	74.4	−3.25		10	168	11.5	8.2	−2.5
	5	156	4.2	21.8	−1		11	196	20.7	14.4	−3.0
	[5] 6	----	----	----	----	4336	1	182	182.0	128.8	−2.5
4331	1	126	40.0	228.0	−1.75		2	189	189.0	134.4	+4.0
	2	126	10.2	58.2	−2.25		3	210	210.0	145.6	+3.75
	3	126	20.6	117.7	−2.75		4	245	245.0	173.6	+1.25
	4	126	8.0	45.7	−2.75		5	245	192.6	131.5	0
	5	108	4.5	25.9	−1		6	266	120.0	79.5	−.5
	[6] 6	----	----	----	----		[7] 7	152	69.7	46.0	----

[1] + indicates gain in weight; −, loss.
[2] Quantity estimated, as feed was spilled.
[3] Pig died at the end of 5 days of this week.
[4] Pig died at the end of 3 days of this week, during which time animal would not eat.
[5] Pig died at the end of 4 days of this week, during which time animal would not eat.
[6] Pig died at the end of 2 days of this week, during which time animal would not eat.
[7] Pig died at end of 4 days of this week.

TABLE 2.—*Summary of results of sodium selenite feeding experiment*

Pig No.	Initial weight	Period from beginning of experiment to death of pig	Final weight	Change in weight [1]	Total dry feed eaten	M/10 solution of sodium selenite per ounce of feed	Total selenium eaten	Selenium in grain feed	Results
	Pounds	*Days*	*Pounds*	*Pounds*	*Pounds*	*Centimeters*	*Grams*	*P. p. m.*	
4326 (control)	38.5	[2]	[3] 75.0	+36.5	151.8	0	0	0	
4327 (control)	30.5	[2]	[3] 64.0	+33.5	133.4	0	0	0	
4328	33.5	46	20.5	−13.0	7.1	1.4	1.14	392	41 days after experiment began, pig was very weak, had diarrhea. Post-mortem examination showed gastroenteritis; feet and hair normal.
4329	36.5	10	32.25	−4.25	1.5	1.4	.26	392	No symptoms of selenium poisoning; apparently died of acute poisoning.
4330	42.0	38	30.5	−11.5	6.0	.7	.49	196	Marked weakness after 14 days; on twenty-eighth day loss of hair from back. Post-mortem examination showed complete loss of hair on back and some above feet; swelling of coronary band, especially on front feet.
4331	26.0	36	15.5	−10.5	5.2	.7	.48	196	Marked weakness after 27 days. Post-mortem examination showed gastroenteritis; hair and feet normal.
4332	49.5	66	24.0	−25.5	31.2	.17	.66	49	Loss of hair and break in coronary band, after 57 days, on all 4 feet. Post-mortem examination showed loss of hair, break in coronary band, ⅛ inch, on all 4 feet; edema around joints, no erosions; bone marrow of femur red and gelatinous.
4333	38.5	99	20.0	−18.5	26.5	.17	.58	49	Loss of hair on back after 37 days. Post-mortem examination showed loss of hair on back; no foot lesions; yellow gelatinous exudate around and in joints; no erosions.
4334	36.0	79	22.5	−13.5	37.7	.087	.42	24.5	Loss of hair after 27 days; foot lesions appeared later. On post-mortem examination all 4 feet showed separation of skin from hoof, about 1 inch wide; loss of hair on back and around feet; edema around joints but no erosions; marrow of long bones red and gelatinous.
4336	41.0	46	51.5	+10.5	75.5	.087	.84	24.5	No loss of hair or foot lesions. Post-mortem examination showed gastroenteritis.

[1] +indicates gain in weight; −, loss.
[2] Animal lived throughout experiment.
[3] At the end of 63 days.

The first pair of principals received grain containing 392 parts of selenium per million, the second pair 196 p. p. m., the third pair 49 p. p. m., and the fourth pair 24.5 p. p. m. (table 2). The quantity of feed given to the controls was increased each week, whereas the feed for the pigs receiving the grain to which selenium had been added was increased only when the daily ration had been cleaned up regularly in the preceding week. The moist feed was kept before the pigs for 3 to 4 hours, when any feed remaining in the box was weighed and discarded. To determine how much selenium had been consumed by each pig, the weight of the feed left in the box was deducted from that given and the selenium content calculated on the basis of the feed eaten. It was not possible to calculate definitely the quantity of feed left in some cases because of the box having been overturned.

EXPERIMENTAL RESULTS

Feeding data and changes in weights of the pigs, by weekly periods, are shown in table 1. Summaries of the results of the tests are shown in tables 2 and 3.

TABLE 3.—*Summary of the results of analyses,*[1] *for selenium content, of principal body parts and fluids of pigs 4328, 4329, 4332, and 4336 (principals) and control pig 4327*

[Results given in terms of per million of selenium parts, based on weight of samples of body parts and fluids taken on post mortem]

Body part or fluid	Selenium content[2] of indicated body part or fluid of—				
	Pig 4328	Pig 4329	Pig 4332	Pig 4336	Pig 4327
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Bile		3	T	6.0	
Blood (clots from heart and larger arteries)	0.5	5.0	2.0	0.2	0
Bone (left femur)	1.0		7.0	T	0
Bone marrow of left femur	T		2.0	0	0
Brain				0	
Hair	0			0	
Heart (muscular tissue only)	T	T	5.0	0	0
Hoof (horny portion only)	20.0		8.0	8.0	0
Hoof joint			.3		
Intestines (large) and contents	1.5	38.5	8.0	[3] T	0
Intestines (small) and contents	1.0	3.5	2.0	1.0	0
Kidney	13.0	4.0	25.0	3.0	0
Leg joint (hock)			4		0
Leg muscle	0		2.0	0	0
Liver	3.0	3.0	10.0	4.0	0
Lungs	T	1.5	2.0		0
Skin (free of hair and dirt)	1.0		T	.8	
Spleen	0	T	T	1.5	0
Stomach (washed free of contents)		0		.5	[4] 0
Testicles			0		
Urine (taken from bladder)	0.1		3.0		0

[1] The methods of analysis used by H. C. Dudley in making these determinations are described in publications by Dudley and Byers (*3*) and Robinson and co-workers (*8*).
[2] T = Trace.
[3] Contained large number of roundworms.
[4] Stomach with contents examined.

CONTROL ANIMALS

Pigs 4326 and 4327, the two control animals, remained normal for the duration of the experiment. Pig 4327 was killed to compare the weights of the organs with those of one of the principals, No. 4332, and also to determine whether or not selenium was present in the tissues. No selenium was found in pig 4327, as shown in table 3.

PRINCIPAL ANIMALS

PIG 4328

Pig 4328 ate relatively little feed after the second day of the experiment and became progressively weaker. It was unable to stand after 41 days and death occurred in 46 days. No clinical evidence of selenium poisoning, such as loss of hair along the back or around the feet, or foot lesions, was observed at any time. On post-mortem examination the only lesions noted were an acute gastroenteritis.

In the histological examination of the liver, very little capillary engorgement was found, but there was considerable deposition of bile pigment, and in some areas a noticeable increase in the interstitial tissue as well as a limited leucocytic and round-cell infiltration. A very large percentage of the liver cells showed various stages of fatty degeneration and marked vacuolation. In some lobules the degenerative changes were more marked around the central veins, whereas in others the changes were not confined to any particular part. Whole columns of cells in some lobules showed practically complete degeneration and disintegration of the cytoplasm, only the cell wall and occasionally the nucleus remaining.

In the kidney, thrombi were observed in a number of the larger veins and there was also some capillary engorgement, as well as evidence of edema, and many of the glomeruli were greatly shrunk or atrophied. The kidney epithelium showed indications of more or less degeneration throughout, but the more marked changes were confined to certain groups of tubules, in many of which complete desquamation of the epithelium had occurred.

In the skin the principal changes noted were in the hair and hair follicles. These consisted of well-marked atrophy, and in a number of follicles, also degeneration which appeared to involve the hair sheath. There were more or less disintegration of the cellular elements and formation of small clumps of fragments, which stained deeply with eosin, suggestive of hyaline change. Heavy deposits of green pigment were noted in a number of the hair papillae.

PIG 4329

Pig 4329 stopped eating 7 days after the experiment began and was in a moribund condition 10 days later, when it was killed. No clinical evidence of selenium poisoning was noted at any time.

Post-mortem examination showed that the peritoneal cavity contained about 50 cc of straw-colored fluid and the pleural cavity a similar quantity. No other changes were observed. No histological examination was made.

PIG 4330

Pig 4330 ate feed very sparingly, showed marked weakness 14 days after the beginning of the experiment, and began to lose hair along the back 14 days after weakness appeared. Denudation was complete 7 days after the hair began to fall, and the pig succumbed 38 days after the beginning of the experiment.

Post-mortem examination showed that the hair had entirely disappeared along the back from the neck to the tail, for a width of about 3 inches, and there was a marked loss of the white hair around the feet. A distinct swelling of the coronary band, similar to the earlier

foot lesions of so-called alkali disease, was present in the front feet and dew claws, and to a smaller extent in the hind feet (fig. 1). The internal organs appeared normal, with the exception of the liver, in which the lobules were slightly paler than normal and were quite prominent.

On histological examination of the liver, it was found that the changes varied in different lobules, from a slight cloudiness of the cytoplasm to complete degeneration of the cells. In some lobules practically all the cells showed extreme cloudy swelling, and in a few lobules engorgement with blood was found in the degenerated centers. Cell vacuolation was very slight and only a small quantity of pigment was noted. There was practically no increase in the interstitial tissue.

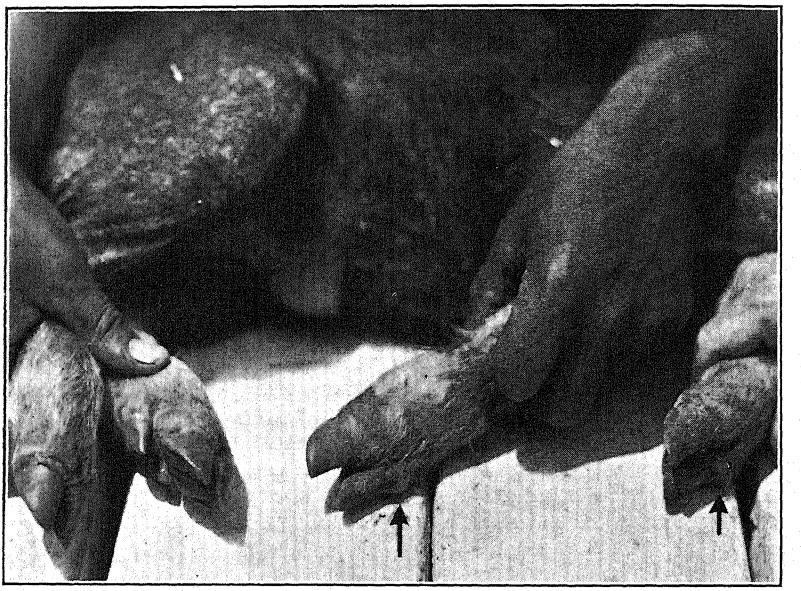


FIGURE 1.—Swelling of the coronary band of the feet of pig 4330. Note particularly swelling of front feet, as indicated by arrows.

In the kidney, little evidence of circulatory disturbance was found. The entire kidney epithelium appeared to be more or less involved in the degenerative changes, which consisted of extensive cloudiness and in certain of the tubules complete degeneration of the cells. In some areas noticeable vacuolation of the degenerated cells was observed. Atrophic changes were noted in many of the glomeruli, and a number of tubules contained hyalin casts.

In the spleen, small degenerated areas were observed in which the cells were undergoing fatty changes.

PIG 4331

Pig 4331 ate little after the second day of the experiment, and 26 days later had difficulty in controlling the hind legs. No loss of hair or changes in the feet were noted. The animal died 36 days after the experiment began.

Post-mortem examination showed severe gastroenteritis, but no macroscopic changes were observed in the other organs.

On histological examination of the liver the sections showed capillary engorgement throughout with some evidence of edema. Although there was more or less cloudiness of the liver cells with definite degeneration of individual cells or small groups of cells, the degenerative changes were not so well marked nor so extensive as those observed in the livers of other animals. There were limited leucocytic and round-cell infiltration and also some increase in the interlobular connective tissue.

In the kidney, the epithelium showed a cloudy swelling in many of the tubules, loss of nuclei, and limited fatty changes in the cell protoplasm. There was some desquamation of the tubules, and a number of the tubules contained casts. Some indication of edema was found, and a considerable number of glomeruli showed more or less atrophy. Little pigment was present in this case.

Pig 4332

In pig 4332 there was marked loss of hair, about 2 inches wide, from the poll to the tail, 57 days after the experiment began, and also a distinct break in the coronary band on all four feet. Later the separation of the coronary band from the wall of the hoof became much more pronounced, permitting considerable mobility of the hoof. The animal died 66 days after the experiment began.

Post-mortem examination showed the carcass to be in very poor condition. The hoofs on all four feet showed distinct breaks, about one-eighth inch wide, around the coronary band. The cervical lymph glands were congested, and the muscles in the throat region appeared to be edematous. The heart was flabby, but the lungs revealed no macroscopic lesions. A mild gastritis was present. The liver seemed smaller than usual, the lobules were prominent, and some light-colored areas, the size of a pea, were present. Nothing unusual was noted in the spleen, kidneys, and bladder. The tissues around the joints appeared edematous, but no erosions were found upon examination. The bone marrow of the femur was red and gelatinous, similar to the condition seen in animals affected with so-called alkali disease.

Post-mortem changes in the liver rendered the histological examination somewhat unsatisfactory. Many of the liver cells were undergoing fatty changes, with vacuolation of the cytoplasm, but complete cell destruction was apparently limited to individual cells or small groups of cells. The outstanding change was the extensive proliferation of the interstitial tissue, the capsule of the organ being very much thickened and the interlobular connective tissue greatly increased. Bile pigment was found in many of the cells.

On histological examination of the kidney, capillary congestion and degenerative changes which were more advanced in certain groups of tubules were found in this organ. There was desquamation of the tubular epithelium and in a few tubules practically complete destruction of the epithelium. In some areas fatty changes with cell vacuolation were noticeable. Small pigment deposits were noted.

Examination of the spleen showed little deviation in this organ from the normal.

Weights of the organs of selenium-fed pig 4332 are compared with the weights of the organs of control pig 4327 in table 4.

TABLE 4.—*Comparative weights of organs of selenium-fed pig 4332 and control pig 4327*

Organ	Weight of organs of—		Organ	Weight of organs of—	
	Pig 4332	Pig 4327		Pig 4332	Pig 4327
	Grams	*Grams*		*Grams*	*Grams*
Heart	100	115	Liver	180	715
Kidneys	90	135	Spleen	20	50
Lungs	340	420	Testicles	50	220

At the beginning of the experiment, as shown by table 2, pig 4327 weighed 30.5 pounds and pig 4332 weighed 49.5 pounds, whereas at the conclusion of the experiment pig 4327 weighed 64 pounds and pig 4332 weighed only 24 pounds. Pig 4327 therefore had gained 33.5 pounds, whereas pig 4332 had lost 25.5 pounds. In pig 4332 the lower weights of the heart, lungs, kidneys, and spleen (table 4) are probably due to the general loss in weight. In the case of the liver and testicles, however, the differences in the weights of the organs seem too great to be explained on this basis.

PIG 4333

Pig 4333 (fig. 2, *C*) began to lose hair along the back 37 days after the beginning of the experiment. No involvement of the feet was noted at any time. The animal died 99 days after being placed on experiment.

Post-mortem examination showed that the skin was denuded of hair along the spinal column to the tail and there was icterus of the skin and sclera. The heart was flabby and the fat was gelatinous. Some inflammation was present at the pyloric end of the stomach. The liver showed hard, hobnaillike lesions. In the kidneys, the pelvis and calyxes were filled with a gelatinous exudate. The spleen and intestines appeared normal. The joints were enlarged owing to a yellow, gelatinous exudate in the surrounding tissue, but no erosions were found on the articular surfaces. Histological examination was not made.

PIG 4334

The hair along the back of pig 4334 was loose 27 days after the experiment began, and 18 days later an area of several inches on either side of the spinal column, from the head to the tail, was practically denuded (fig. 2, *B*). In about 2 months a break appeared in the coronary band of the feet, becoming progressively wider until the separation attained a width of about 1 inch in the front feet (fig. 3). The animal lived 79 days.

On post-mortem examination all four feet and dew claws showed separation of the hoof to about 1 inch, leaving a raw area. Apparently the tissue from which the horn develops had been impaired since no new hoof was developing. There was loss of hair around the feet and down the back. The carcass was in poor condition and no

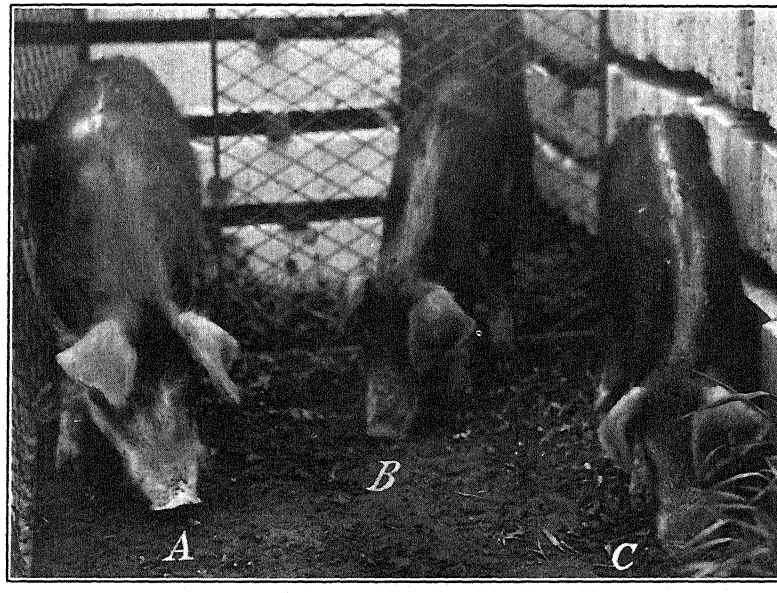


FIGURE 2.—Pigs 4333 (*C*), 4334 (*B*)—two of the principals used in the experiment—and 4326 (*A*), one of the controls. Pig 4333 shows loss of hair along the backbone, typical of selenium poisoning. Pig 4334 shows a small loss of hair, principally over the shoulders. Photograph was taken toward the end of the experiment.



FIGURE 3.—Front feet of pig 4334, which show a characteristic lesion of selenium poisoning in the marked separation of the hoof from the skin at the coronary band.

fat was present, a gelatinous substance having replaced this tissue. The heart was flabby and the fat had a gelatinous appearance. There was gastritis but no enteritis. The spleen appeared normal, but the liver was very hard and small and in one lobe the lobules stood out prominently. The bladder had a yellowish exudate on the mucous surface, which was thickened and showed several small hemorrhages. No erosions were found in the joints, but a gelatinous material was present around some of them. The marrow of the long bones was reddish and gelatinous but not to so marked a degree as in some of the other pigs.

Histological examination of the liver showed a well-marked degeneration of the liver cells, which appeared to be more extensive in some portions of the organ than in others. A definite central necrosis was present in a number of lobules, and these necrotic areas had been invaded by polymorphonuclear leucocytes. Considerable bile pigment, mostly intracellular, had been deposited in the organ, and there was rather extensive proliferation of the interstitial tissue. No other tissue was taken from this animal.

Pig 4336

Pig 4336 never showed any clinical symptoms of selenium poisoning other than a slight loss of appetite 10 days before death, which occurred rather suddenly. This animal lived 46 days.

On post-mortem examination the only lesion observed in the animal was gastroenteritis.

On histological examination of the liver, most of the interlobular veins showed engorgement and a limited number of the lobules showed capillary congestion. Many of the lobules contained areas, varying in size, of degenerated cells in many of which vacuoles were noted. Central necrosis appeared in a few lobules. A small quantity of bile pigment was observed, and there was well-marked proliferation of the interstitial tissue, both interlobular and intralobular.

In the kidney there was more or less blood engorgement throughout the organ. Although much of the tubular epithelium showed cloudy swelling and some desquamation in addition, the more advanced degenerative changes were limited to individual tubules or small groups of tubules. These changes paralleled somewhat those noted in the kidney of pig 4331. There was considerable deposition of pigment also.

In the skin some atrophic changes appeared in certain of the hair follicles.

With regard to the deposition of selenium in the tissues, table 3 shows that the results of the chemical analysis, for selenium content, of the body parts or fluids of pigs 4328 and 4336 are very similar with the exception of the kidney and hoof. Both of these animals lived 46 days, but the quantity of selenium consumed by pig 4328 was 1.14 g as compared with only 0.84 g consumed by pig 4336 during the same period. This difference, then, might account for the larger quantity of selenium found in the kidney and hoof of pig 4328.

DISCUSSION

The clinical symptoms shown by swine affected by the so-called alkali disease in certain areas in the north-central Great Plains States are manifested by a loss of hair and a marked alteration in the growth of the horn of the hoof, which apparently begins as an inflammation of the coronary band and results in an interruption of the normal growth of horn. This is followed by a distinct break in the horn with a marked separation between the new and the old horn. In addition to these symptoms, diarrhea, stiffness, posterior paralysis, marked loss of condition, and death have been observed occasionally following the feeding of alkali grain to swine. Post-mortem examination of several field cases showed a gelatinous condition around the joints, and the marrow of the long bones was reddish, soft, and gelatinous.

The symptoms and lesions produced in the experimental pigs by the ingestion of sodium selenite are similar to those seen in affected swine in the localities where this condition prevails and to those produced by feeding toxic corn outside the area, as reported in a previous paper (*9*).

Of the eight pigs fed sodium selenite, four showed a definite loss of hair most marked along the back; two of the four had distinct breaks in the feet between the skin and hoof at the coronary band; and one had marked swelling of the coronary band. These pigs received the following number of parts of selenium per million parts of grain: 196 parts by pig 4330; 49 parts by pig 4332; 49 parts by pig 4333; 24.5 parts by pig 4334. The remaining four animals showed more or less evidence of enteritis, either clinically or at post mortem. One had diarrhea and several of the others showed marked weakness. All the animals, with the exception of pig 4336, lost weight and all died within 10 to 99 days after the feeding of sodium selenite was begun. Their loss in weight was due, at least in part, to the small quantities of feed consumed. Early in the experiment these animals showed a marked aversion to selenium-treated grain, a characteristic which has been noted in pigs consuming grain in which selenium occurs under natural conditions.

There appears to be considerable variation in the tolerance of the individual pigs to the action of selenium. This is evidenced both by the apparently acute poisoning of pig 4329, which lived only 10 days after consuming 0.26 g of selenium, and by the appearance of clinical symptoms of the disease in some animals (pigs 4330, 4332, 4333, and 4334), which received a larger total quantity of selenium and lived much longer than pig 4329. This difference is further illustrated in the case of three other animals (pigs 4328, 4331, and 4336) which died without showing typical clinical symptoms, although the quantity of selenium consumed and the period of survival were more or less comparable to those of the four pigs which developed lesions. In pig 4332, which survived 66 days, it appears that there was continued deposition of selenium in the various body parts or fluids, in spite of the greatly reduced quantities of the element ingested in the latter part of the feeding period (table 1). Inasmuch as pig 4329 appears to have died of acute poisoning, the differences in the selenium content of the blood and intestinal tract may be due to the large quantity of the salt taken in during the short period of survival (table 1).

SUMMARY

Experiments were made to determine the relationship of selenium to the so-called alkali disease and the effects of selenium in feed given to pigs at different levels over a period of time. Eight pigs about 4 months old were divided into four groups and were fed an adequate grain ration to which selenium in the form of sodium selenite was added in the proportions of 392, 196, 49, and 24.5 parts per million, respectively. Four, or 50 percent, of these animals showed clinical symptoms similar to those seen in natural cases of so-called alkali disease, manifested by a loss of hair and interference with the growth of the horn of the hoof. All the animals died in from 10 to 99 days and showed post-mortem lesions similar to those seen in the so-called alkali disease. Two other pigs, fed the same grain to which no sodium selenite had been added, made normal gains in weight during the feeding period and remained healthy at all times.

The pigs fed the sodium selenite ate very sparingly, leaving a large part of their daily ration untouched, and their loss in weight may be attributed, at least in part, to this factor. The two control pigs kept under identical conditions and receiving the same feed except for the addition of sodium selenite, made gains in weight and remained healthy at all times. After the first few feedings there was evidence of a marked aversion to the feed to which selenium was added. After refusing this feed, animals readily ate normal feed on several occasions when it was offered to them. Aversion to grain in which selenium occurs under natural conditions has been reported from the field.

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THE MEASUREMENT AND INHERITANCE OF SCAB RESISTANCE IN SELFED AND HYBRID PROGENIES OF POTATOES[1]

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INTRODUCTION

In any attempt to breed potatoes (*Solanum tuberosum* L.) for scab resistance one is immediately faced with the problem of accurately measuring the observed differences. The relative usefulness of different methods of measurement can be ascertained by comparing the consistency with which they present evidence of significant inherited differences in scab resistance. In this paper is presented a study of the technique of measuring differences in scab reaction between seedling progenies, together with the relation of selfed and hybrid progenies in respect to these differences.

MATERIALS AND METHODS

The tests for scab resistance were made on a plot of peat soil at the Coon Creek experimental farm near Anoka, Minn. The soil of a greater portion of the plot is rather heavily infested with *Actinomyces scabies* (Thax.) Güss., and had grown scabby crops of potatoes for 4 successive years. In 1936, when it was necessary to increase the size of the plot, some soil was included on which potatoes had not been grown in recent years and which was somewhat less heavily infested.

The seedling progenies tested included 33 selfed lines and 27 crosses. The number of seedlings in the different families ranged from 25 to 350. Each family was planted in approximately equal numbers in three randomized blocks. Many of the parents had heen tested as clones, and differences had been observed which appeared to be significant. The progenies studied were started from seed in September and the seedlings were grown to maturity in the coldframe and greenhouse in the fall of 1935. Small tubers ranging from ¼ to 1 inch in diameter were harvested. These were planted in the field test plots in the spring of 1936. The small size of the tubers planted, together with unusually hot and dry weather during midsummer, resulted in late setting and a light crop of relatively small tubers. In general, the scab infection on the seedling families was less than that on the common varieties and older selections in the test plot. This relatively light infection tended to decrease the range of variability in the

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amount of scab, thus increasing the importance of accuracy in measurement.

When the crop was harvested the tubers from each hill were placed in a separate paper bag and taken to the laboratory for a more careful examination of scab infection. The tubers were washed before scab readings were made. Each individual tuber was given a rating on the basis of resistance to scab infection according to the following scale:

0=No scab.
1=Very small lesions—very resistant.
2=Small lesions—moderately resistant.
3=Medium-sized lesions—showing some evidence of resistance.
4=Large lesions—no evidence of resistance.

After all of the tubers of a hill had been classified according to the above scale, a scab rating for the hill was obtained by multiplying the number of tubers in each class by the number of the class and dividing the product by the total number of tubers in the hill. Thus each hill received a numerical rating falling somewhere between 0 and 4, inclusive, corresponding to its observed susceptibility to scab; the larger the figure, the greater the susceptibility. The scab rating for a seedling family was obtained by averaging the ratings of the individual hills of the family.

Before the notes were taken, tubers representing each class in the scale were selected and photographed at natural size (fig. 1). All readings were made by one person who had the photographs before him at all times. The seedling families were labeled by row number only and their identity was unknown to the observer, so that there was no opportunity for bias.

It will be noted that in the photographic scale the samples of the more susceptible types of lesion always involve a larger surface area of infection. This correlation was illustrated because it agrees with previous observations and because it was expected and desired that the ratings be influenced to some extent by the amount of surface area infected. The scab rating, however, is based primarily on resistance as manifested in the type of lesion and secondarily on the amount of surface area infected. In other words, a tuber with a single large susceptible-type lesion would be rated as susceptible, but a tuber with a relatively high percentage of its surface area affected with scab composed of small, shallow, resistant-type lesions would be rated as resistant.

Clark, Raleigh, and Stevenson[2] used data based on the amount of surface area of the tubers affected with scab in arriving at a numerical "scab index" in a test of varietal resistance to scab. The type of lesion is given as supplementary data by these workers and is not used in computing the scab index. In the present method the results of both criteria of measurement are combined in a single numerical rating, thereby facilitating the use of all data in statistical analyses. The end result of these two methods may not differ greatly, because there is obviously some agreement between resistance as indicated by the type of lesion and as indicated by the amount of surface area affected in a given test. In general, the more resistant the

[2] CLARK, C. F., RALEIGH, W. P., and STEVENSON, F. J. BREEDING FOR RESISTANCE TO COMMON SCAB IN THE POTATO. Amer. Potato Jour. 13: 256–259. 1936.

lesion type, the smaller is the area affected. However, striking exceptions are often observed. It is believed that a single, large, susceptible-type lesion on a tuber is more likely to indicate susceptibility than resistance. Using the surface-area method of arriving at a scab index, such a tuber would weight the results on the side of re-

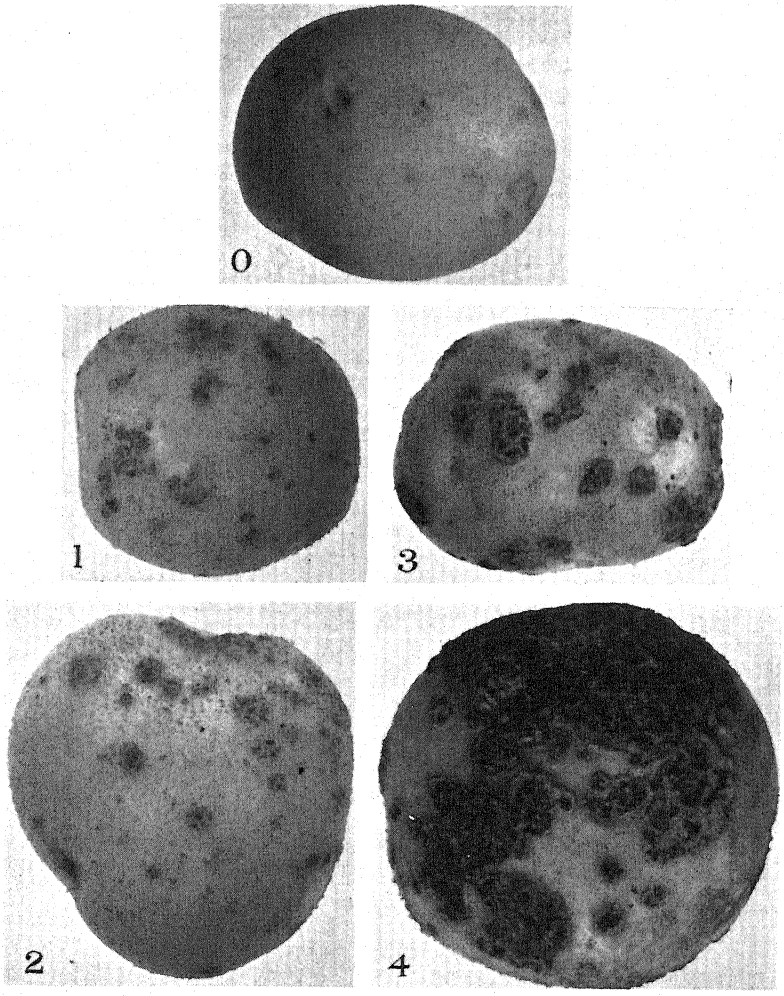


FIGURE 1.—Five tubers illustrating the scale used in rating the relative scab resistance of potatoes tested, those shown representing the minimum limits for each class: 0, No scab; 1, very small lesions—very resistant; 2, small lesions—moderately resistant; 3, medium-sized lesions—showing some evidence of resistance; 4, large lesions—no evidence of resistance. × 4/5.

sistance and constitute a source of error that could be eliminated only by numerous replications and checks that are not possible in many types of test.

Four different methods of classifying the material were used. The first method included the ratings on all tubers regardless of size. The second method included the ratings only on those tubers larger

than 1 inch in diameter. It was thought that many of the smaller tubers would have escaped infection and would constitute a source of error.

In the third method each hill was given a rating corresponding to the most susceptible-type lesion found on any tuber in the hill. The justification for this method is based on the theory that if one tuber in the hill is susceptible to a given degree all others in the hill are potentially susceptible to an equal degree but have escaped infection. If this method should prove reliable it would greatly simplify the task of testing for scab resistance.

The fourth method consisted in rapidly rating each hill into one of the five classes on the basis of the predominant type of scab lesion among all the tubers of the hill. An attempt was made to have this method somewhat comparable to what would be accomplished if the individual hills were rated in the field at harvest time.

EXPERIMENTAL RESULTS

METHOD OF MEASURING SCAB

The data obtained by these various methods of classification are presented in tables 1 and 2. In table 1 are given the data on the inbred families; the data on the crosses are given in table 2. The data are separated into two tables merely for convenience. The analysis of variance was applied to the data as a whole.

TABLE 1.—*Mean scab ratings of 33 inbred families as measured by the 4 different methods*

Family	All tubers	Large tubers	Highest scab	Predominant scab type
81-1	1.58	1.70	2.21	1.48
11-1-3-2	1.34	1.30	2.12	1.60
82-1	1.06	1.26	2.06	1.37
29-14	1.08	1.16	1.82	1.34
29-13	1.00	1.21	1.48	1.46
11-1-2-1	.78	1.01	1.57	1.00
13-1	.54	.96	1.40	1.24
11-8-1-9-1	.72	.93	1.48	1.22
3-2-4	.82	.92	1.61	1.32
34-1-1	.84	.90	1.59	1.13
12-2-4-1-7	.74	.82	1.56	1.29
9-1	.70	.88	1.40	1.14
29-13-5	.51	.81	1.50	1.12
40-2-2	.70	.80	1.42	1.14
10-4-2	.51	.78	1.34	1.03
1-4-2	.49	.78	1.56	1.20
82-8	.51	.76	1.33	1.00
82-6	.47	.68	1.30	1.04
11-1-2-1-1	.42	.64	1.31	1.08
82-10	.49	.64	1.43	1.10
82-11	.45	.58	1.21	1.14
82-4	.59	.68	1.37	1.06
82-2	.56	.67	1.70	1.26
11-8-1-9-3	.54	.63	1.32	1.08
11-8-1-9-4	.45	.59	1.36	1.10
11-1-2-2-4	.44	.56	1.26	1.02
5-10-1	.46	.52	1.33	1.07
14	.36	.52	1.15	1.03
1-1	.35	.50	1.14	1.00
1-4-1	.32	.48	1.22	1.00
21-2-2	.36	.48	1.48	1.00
15-1	.31	.42	1.06	1.00
11-1-25	.24	.36	1.00	1.00
S. E. of difference	.167	.190	.214	.241
Difference required for significance	.33	.38	.43	.48

Because of the loss of 1 replication of 16 families before notes were taken, there were 3 replications for only 44 of the families. The data were analyzed separately for two and three replications. The results of these analyses are given in table 3. When the variance for error for three replications is compared with that for two replications it is seen that the third replication added relatively little to the reliability of measurement. This is probably because the third replication was planted on soil that was less heavily infested with scab. In view of this fact, and because it was desired to use the data on certain families with only two replications, the data based on two replications only were used in further consideration of the results of the experiment.

TABLE 2.—*Mean scab ratings of 27 hybrid families as measured by the 4 different methods*

Cross No.	Parents	All tubers	Large tubers	Highest scab	Predominant scab type
123.35	Triumph × 4-25-7-1	1.54	1.67	2.54	1.80
121.35	Warba × 11-1-3-2	1.17	1.28	2.24	1.56
119.35	Warba × 12-2-4-1-7	1.00	1.16	1.78	1.32
31.35	11-1-3-2 × 40-4-2-5-1	.98	1.14	1.94	1.46
125.35	Triumph × 40-2-2-2-1	1.04	1.12	1.84	1.00
194.35	Early Ohio × 12-2-4-1-7	.80	.99	1.84	1.40
118.35	Warba × 5-10-1	.78	.94	1.60	1.24
111.35	Early Ohio × 5-10-1	.80	.91	1.61	1.22
112.35	Early Ohio × 41-1-1-7-6-1	.80	.89	1.56	1.26
27.35	11-1-2-1 × 39-3-1-1-5	.76	.88	1.66	1.27
124.35	Triumph × 5-10-1	.78	.87	1.66	1.23
107.35	82-10 × 12-2-4-1-7	.76	.84	1.59	1.22
148.35	Russet Rural × 5-10-1	.70	.84	1.57	1.34
164.35	Erstling × 40-2-2	.72	.84	1.72	1.10
32.35	11-1-2-2-4 × 21-2-2	.64	.74	1.53	1.00
172.35	116.30-1-31 × 4-25-7-1	.58	.74	1.64	1.29
165.35	Jubel × 5-10-1	.64	.73	1.26	1.14
39.35	11-8-1-9-4 × 21-2-2	.57	.72	1.62	1.26
104.35	82-4 × 12-2-4-1-7	.74	.72	1.71	1.25
1.35	4-9-3-1-1-1-1-1 × 5-10-1	.56	.70	1.62	1.08
84.35	74-1 × 21-2-2	.55	.64	1.54	1.16
173.35	116.30-1-31 × 5-10-1	.60	.64	1.10	1.26
113.35	Early Ohio × 4-9-3-1-1-1-1-1	.64	.64	1.66	1.20
9.35	4-25-6 × 5-10-1	.48	.55	1.49	1.20
5.35	4-9-3-1-1-1-1-1 × 82-10	.38	.52	1.40	1.06
167.35	Arnica × 5-14-8-1	.32	.38	1.08	1.00
166.35	Jubel × 5-14-8-1	.21	.30	1.03	1.18
	S. E. of difference	.167	.190	.214	.241
	Difference required for significance	.33	.38	.43	.48

TABLE 3.—*Summary of analyses of variance of data on scab resistance of 60 seedling families based on 4 different methods of measuring scab resistance, and on 2 or on 3 replications*

THREE REPLICATIONS

Variation due to—	Degrees of freedom	Variances			
		All tubers	Large tubers only	Mean highest scab rating	Predominant scab type
Families	43	[1] 0.215	[1] 0.216	[1] 0.283	[1] 0.065
Error	86	.029	.033	.043	.026

TWO REPLICATIONS

Families	59	[1] 0.168	[1] 0.165	[1] 0.194	0.057
Error	59	.028	.036	.058	.046

[1] The value of F exceeds the 1 percent point.

It is evident from table 3 that significant differences in scab susceptibility were revealed by all four methods of measurement, but the most effective method was that based on all tubers, closely followed by that based on large tubers only. The ratings based on the highest scab type gave significant differences between families, while the method of rating on the predominant scab type proved unsatisfactory.

A further test of the efficiency of the different methods of scab measurement was obtained by determining the degree of correlation between the mean scab ratings of related F_1 progenies. Theoretically, one would expect a positive correlation of the mean scab ratings of related progenies if the methods of measurement are reliable. Fourteen selections or varieties entered into one or more crosses. The coefficient of correlation for these related progenies was determined for each of the methods of recording the scab data. The coefficients of correlation were as follows:

All tubers	0. 59	Highest scab type	0. 34
Large tubers	. 50	Predominant scab type	. 08

The coefficient based on all tubers and on large tubers is highly significant, exceeding the 1-percent point. That based on the highest scab type exceeds the 5-percent point and is also significant, but that based on the predominant scab type is not significant. An analysis of variance of the mean scab ratings within and between these related groups as given in table 4 shows essentially the same relationship.

TABLE 4.—*Analysis of variance of scab ratings within and between groups of related F_1 families*

Variation—	Degrees of freedom	Variance			
		All tubers	Large tubers	Highest scab	Predominant scab type
Between groups	11	[1] 0. 1636	[1] 0. 1797	[1] 0. 2222	0. 0398
Within groups	25	. 0309	. 0357	. 0459	. 0221

[1] The value of F exceeds the 1 percent point.

The tubers in this experiment were small. That larger tubers would increase the accuracy of rating is suggested by a comparison of the selfed families with the hybrid families given in table 5. The 33 selfed lines yielded on an average about 50 percent less than the hybrids. Since the average number of tubers per plant did not differ greatly for the two groups, most of the difference in yield may be attributed to size of tubers. The average mean scab ratings for the two groups were the same only where none but the largest tubers in the hill were considered. For the other three methods of rating, the average scab ratings of the selfed lines are less than those of the hybrid families. Although these differences are not large, they suggest that the smaller tubers in the selfed lines, by escaping infection, may have caused a relatively lower mean scab rating of the selfed lines as compared with the hybrid families. If this were true, all other things being equal, one would expect the average mean scab

rating of the selfed lines to be considerably lower than that of the hybrid families. However, since many of the hybrid families were from crosses made for the specific purpose of introducing scab resistance, it is probable that this tended to equalize the average mean scab rating of the two groups. Additional evidence as to the reliability of the methods may be obtained from a consideration of tables 6, 7, 8, and 9. In tables 6, 7, and 8 the mean scab ratings of the progenies are listed in order of susceptibility on the basis of data on the large tubers. It will be noted in each table that the progenies do not retain the same relative position by each of the four methods of rating, but the general trend is the same. The rating of the progenies by the "predominant scab type" method is not in agreement with that obtained by the other methods. This method, as has been shown, is also the least reliable.

TABLE 5.—*Yield and scab ratings of the selfed lines and the hybrid families*

Item	Families	Mean number of tubers per plant	Mean yield per hill	Mean scab ratings			
				All tubers	Large tubers	Highest scab	Predominant scab type
	Number		*Grams*				
Selfed lines	33	4.10	86	0.65	0.797	1.45	1.15
Hybrid families	27	4.99	169	.72	.792	1.62	1.24
Difference required for significance				.060	.069	.078	.088

In table 9 those crosses that were included in the test and in which the scab resistance of the parents is known, are arranged in six groups on the basis of the susceptibility of the parents. The scab ratings of the progenies of these crosses are in striking agreement with the known scab resistance of the parents. It is difficult to believe that such close agreement could have been possible if the methods of measuring scab resistance had not been reasonably accurate.

HEREDITARY DIFFERENCES IN RESISTANCE BETWEEN PROGENIES

It can be shown that the observed differences in mean scab rating are hereditary by grouping together those hybrid families with a common parent and comparing the variance in mean scab rating within and between these groups of related families. Twelve groups, comprising 37 hybrid families, are available for comparison. In table 4 are given the variances obtained within and between groups by each of the four methods of classification. A significant difference was found between groups of related families by the "all tubers," "large tubers," and "highest scab" methods of rating. The results indicate that the mean scab ratings of the families within the groups were more similar than the ratings of the families in different groups. The uniformity obtained within groups can in part be ascribed to the influence of the common parent for each group of crosses and the significant variances obtained between groups is due to significant differences in breeding behavior for scab resistance between the common parents. The common parents and the average mean scab

rating for each of the 12 groups are given in table 6. It has been established that there remains a significant difference between these groups of related progenies after the removal of the variance due to the unrelated parents within each group (table 4). It is of interest to note that the Triumph, 11–1–3–2, and Warba show no breeding value for scab resistance. On the other hand, the variety Jubel and the selection 5–14–8–1 have a high breeding value, as indicated by the low mean scab ratings of their hybrid progeny. It would be unsafe to draw conclusions on the intermediate groups, although a further analysis of these parents indicates that some of them have considerable breeding value for scab resistance.

TABLE 6.—*The common-parent and the average-mean-scab rating for each of 12 groups of F_1 families*

Group No.	Common parent	F_1 families	Average scab rating of F_1 families			
			All tubers	Large tubers	Highest scab	Predominant scab type
		Number				
1	Triumph	3	1.12	1.22	2.01	1.34
2	11–1–3–2	2	1.08	1.21	2.09	1.56
3	Warba	3	.98	1.13	1.87	1.37
4	12–2–4–1–7	4	.82	.93	1.73	1.30
5	Early Ohio	4	.76	.86	1.67	1.27
6	5–10–1	8	.67	.77	1.49	1.21
7	40–2–2	2	.64	.78	1.67	1.18
8	21–2–2	2	.58	.69	1.54	1.08
9	82–10	2	.57	.68	1.50	1.14
10	4–9–3–1–1–1–1–1	2	.53	.62	1.56	1.11
11	Jubel	2	.42	.52	1.14	1.16
12	5–14–8–1	2	.26	.34	1.05	1.09
Difference required for significance[1]			.167	.190	.214	.241

[1] Between those groups containing more than 2 F_1 families a smaller difference would be required for significance.

TABLE 7.—*Mean scab rating of F_1 progenies of 8 parents, each crossed with line 5–10–1*

Cross No.	Parents	All tubers	Large tubers	Highest scab	Predominant scab type
118.35	Warba × 5–10–1	0.78	0.94	1.60	1.24
111.35	Ohio × 5–10–1	.80	.91	1.61	1.22
124.35	Triumph × 5–10–1	.76	.87	1.66	1.23
148.35	Russet Rural × 5–10–1	.70	.84	1.57	1.34
165.35	Jubel × 5–10–1	.64	.73	1.26	1.14
1.35	4–9–3–1–1–1–1–1 × 5–10–1	.56	.70	1.62	1.08
173.35	116.30–1–31 × 5–10–1	.60	.64	1.10	1.26
9.35	4–25–6 × 5–10–1	.48	.55	1.49	1.20
Difference required for significance		.33	.38	.43	(1)

[1] Not significant.

In the above study it has been shown that, when all the crosses in which a given parent entered are grouped, there are significant differences between the average mean scab rating of these groups. A comparison between the hybrid families within a group having a common parent is given in table 7. These eight hybrid families are taken from group 6, table 6, and consist of crosses between selection 5–10–1 and

eight other parents. It will be noted from table 7 that the largest differences between the mean scab ratings of the hybrid progenies approach twice the standard error. The five varietal parents gave hybrid families with mean scab ratings approaching the order of their behavior as clones in the varietal test plot. It would appear from these results that the method of crossing all individual selections to a common parent tends to bring out existing hereditary differences. However, in these tests it was a less satisfactory method of testing the breeding value of selections for scab resistance than the method of testing the selections in a group of crosses or of testing their selfed progenies.

TABLE 8.—*Mean scab rating of progenies of 10 parents when selfed and when crossed*

Parents	Selfed				Crossed			
	All tubers	Large tubers	Highest scab	Predominant scab type	All tubers	Large tubers	Highest scab	Predominant scab type
11–1–3–2	1.34	1.30	2.12	1.60	1.08	1.21	2.09	1.51
11–1–2–1	.78	1.01	1.57	1.00	.76	.88	1.66	[1] 1.23
12–2–4–1–7	.74	.82	1.56	1.29	.82	.93	1.73	1.30
40–2–2	.70	.80	1.42	1.14	.64	.78	1.67	1.18
82–4	.59	.68	1.37	1.04	.74	.72	1.71	[1] 1.25
82–10	.49	.64	1.43	1.10	.57	.68	1.50	1.14
11–8–1–9–4	.45	.59	1.36	1.10	.57	.72	1.62	[1] 1.26
11–1–2–2–4	.44	.56	1.26	1.02	.64	.74	1.53	[1] 1.00
5–10–1	.46	.52	1.33	1.07	.67	.77	1.49	1.21
21–2–2	.36	.48	1.48	1.00	.60	.69	1.54	1.08
Difference required for significance	.33	.38	.48	[2]	.33	.38	.43	[2]

[1] 1 cross only.
[2] Not significant.

The relation of the mean scab ratings of selfed lines with the average mean scab rating of all the crosses in which each line entered is given in table 8. This table shows the mean scab ratings of 10 selfed lines and the average mean scab rating for the hybrid families from each selection. Selfed lines 11–1–3–2 and 11–1–2–1 have a significantly higher mean scab rating than 5–10–1 and 21–2–2. Wider differences exist between the selfed lines than between their hybrid progenies. In the latter a significant difference is shown by 11–1–3–2, which, in both its selfed line and hybrid progenies, gave a high mean scab rating. In this comparison the selfed lines proved much more effective in bringing out significant differences between selections than their hybrid families.

In the studies so far presented the behavior of F_1 families has been compared with that of one parent of the cross. In table 9 the crosses are grouped according to the reaction of the parents to scab. The five groups are as follows: Susceptible × susceptible, susceptible × resistant, intermediate × intermediate, intermediate × resistant, and resistant × resistant. The parent varieties are classed as susceptible or resistant on the basis of 2 or more years' test. The parent selections were placed in the susceptible, intermediate, and resistant classes according to the mean scab rating of their selfed lines as given in table 8. The data show that the mean scab ratings of crosses

between susceptible parents are significantly higher than in the crosses of the susceptible × resistant group. This latter group has a higher scab rating than the group of crosses between intermediate and the intermediate × resistant group. The difference obtained between the latter two groups is insignificant. The crosses between the resistant parents gave a significantly lower average mean scab rating than the other four groups of crosses.

TABLE 9.—*Mean scab ratings of F_1 progenies of 14 crosses grouped according to the susceptibility of the parents*

Group	Type of cross [1]	Cross No.	Parents	Mean scab rating of F_1 progenies			
				All tubers	Large tubers	Highest rating	Predominant rating
1	S × S	121.35	Warba × 11-1-3-2	1.17	1.28	2.24	1.56
2	S × I	119.35	Warba × 12-2-4-1-7	1.00	1.16	1.78	1.32
		194.35	Ohio × 12-2-4-1-7	.80	.99	1.84	1.40
			Mean	.90	1.08	1.81	1.36
3	S × R	124.35	Triumph × 5-10-1	.78	.87	1.66	1.23
		118.35	Warba × 5-10-1	.78	.94	1.60	1.24
		111.35	Ohio × 5-10-1	.80	.91	1.61	1.22
			Mean	.79	.91	1.62	1.23
4	I × I	39.35	11-8-1-9-4 × 40-2-2	.57	.72	1.62	1.26
		104.35	12-2-4-1-7 × 82-4	.74	.72	1.71	1.25
		107.35	12-2-4-1-7 × 82-10	.76	.84	1.59	1.22
			Mean	.69	.76	1.64	1.24
5	I × R	32.35	11-1-2-3-4 × 21-2-2	.64	.74	1.53	1.00
		39.35	11-8-1-9-4 × 21-2-2	.57	.72	1.62	1.26
			Mean	.61	.73	1.58	1.13
6	R × R	167.35	Arnica × 5-14-8-1	.32	.38	1.08	1.00
		165.35	Jubel × 5-10-1	.64	.73	1.26	1.14
		166.35	Jubel × 5-14-8-1	.21	.30	1.03	1.18
			Mean	.39	.47	1.12	1.11
Difference between families required for significance [2]				.33	.38	.43	(3)

[1] S=susceptible; I=intermediate; R=resistant.
[2] A smaller difference would be required for significance between the group means.
[3] Not significant.

SUMMARY

The problem of measuring differences in scab resistance in potatoes was studied, with particular reference to difference between seedling families. A mean scab rating for each family was determined and expressed by the use of a numerical scale which gave a measure of resistance based on the type of lesion and also took into account the amount of surface area affected. By combining the results of both criteria of measurement in a single numerical rating, this scale facilitated the use of all the data in statistical analyses.

Four different methods of measurement were compared in a study of the reaction of 33 selfed lines and 27 hybrid families. In one method each tuber in the hill was classified according to the scale, and from these data an average rating for the hill was determined. In another method the average rating for the hill was based on the readings on

only those tubers larger than 1 inch in diameter. The average of the hill ratings was determined to obtain the mean scab rating of the family. Both of these methods gave very satisfactory results. Two simplified methods of measurement were based on the most susceptible type of lesion and the predominant type of lesion in a hill, respectively, but these were less satisfactory than the other two methods.

Significant differences in mean scab rating were obtained by all four methods of measurement. The most significant differences were obtained by the methods of "all tubers" and "large tubers," and the least significant by the predominant scab type method. In the further analyses of the breeding material the four methods maintained the same relative position as regards their effectiveness in measuring significant differences.

The hereditary nature of the differences in scab resistance found between the seedling families was shown by a greater variance between groups of crosses where each group had a common parent than within these groups. A direct comparison of these groups showed significant differences in reaction to scab and indicated the relative breeding value of the parents. Eight F_1 families having 5–10–1 as a common parent gave differences in mean scab rating which in some cases approached significance, indicating a difference between the unrelated parents in their ability to transmit scab resistance.

A significant difference between the mean scab rating of 10 selfed lines was associated with a similar but smaller difference between the averages of their hybrid families.

When the crosses were grouped according to the reaction of both parents it was found that crosses between susceptible parents gave a significantly higher mean scab rating than crosses of susceptible with resistant parents. The latter crosses had a significantly higher mean scab rating than crosses between intermediates and crosses of intermediate with resistant parents. The crosses between the resistant parents gave a significantly lower mean scab rating than any of the other four types of crosses.

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THE CONTROL OF DANDELIONS IN LAWNS[1]

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INTRODUCTION

The common dandelion (*Taraxacum officinale* Weber) is the most troublesome weed of lawns in the cooler portions of the United States. The flattened leaf rosette and contractile perennial taproot adapt the plant to growth in closely mowed or pastured grasslands, while its ability to withstand drought or unfavorable conditions when once established, and to regenerate new crown buds, even when cut at depths of 8 to 10 cm below the surface, contribute to its permanence.

The growth of the dandelion in sod, and particularly in lawns, prevents the use of cultivation or the usual herbicides as control measures. Deep hand cutting and iron sulphate sprays have been most commonly used, but neither method is effective unless repeated several times at frequent intervals and under favorable conditions.

Work on measures for the control of dandelions was initiated at Ames, Iowa, by Dr. I. E. Melhus and has been pursued intermittently for the last 9 years. The results of some of the earlier experiments have been published by Melhus (*18*)[2] and Arnold (*3*).

The work on dandelion control reported here may be divided into three sections: (1) that concerned with the biology and particularly with the food reserves of the plant; (2) the use of distillates as differential herbicides for the control of dandelions in bluegrass sod; and (3) the ecological factors concerned in the establishment and maintenance of dandelion-free lawns.

THE DANDELION ROOT

Aside from mechanical removal, the eradication of dandelions depends either upon killing the fleshy taproot or so weakening it as to prevent regeneration after cutting or spraying treatments. The taproot system of the plant extends 3 or more feet into the ground, and much of the root system of the older plants extends below the level of the grass roots. This deep rooting is an important factor in the ability of the plant to compete with bluegrass or other sods.

The upper portions of the taproot, which may attain a diameter of 2 cm, contain the abundant xylem and phloem parenchyma characteristic of storage roots. Figure 1, *A* and *B*, shows this abundance of parenchyma, particularly in the phloem. Figure 1, *B*, shows also two features characteristic of older roots; namely, (1) the cutting off and sloughing of the older phloem, and (2) the tendency toward

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[2] Reference is made by number (italic) to Literature Cited, p. 867.

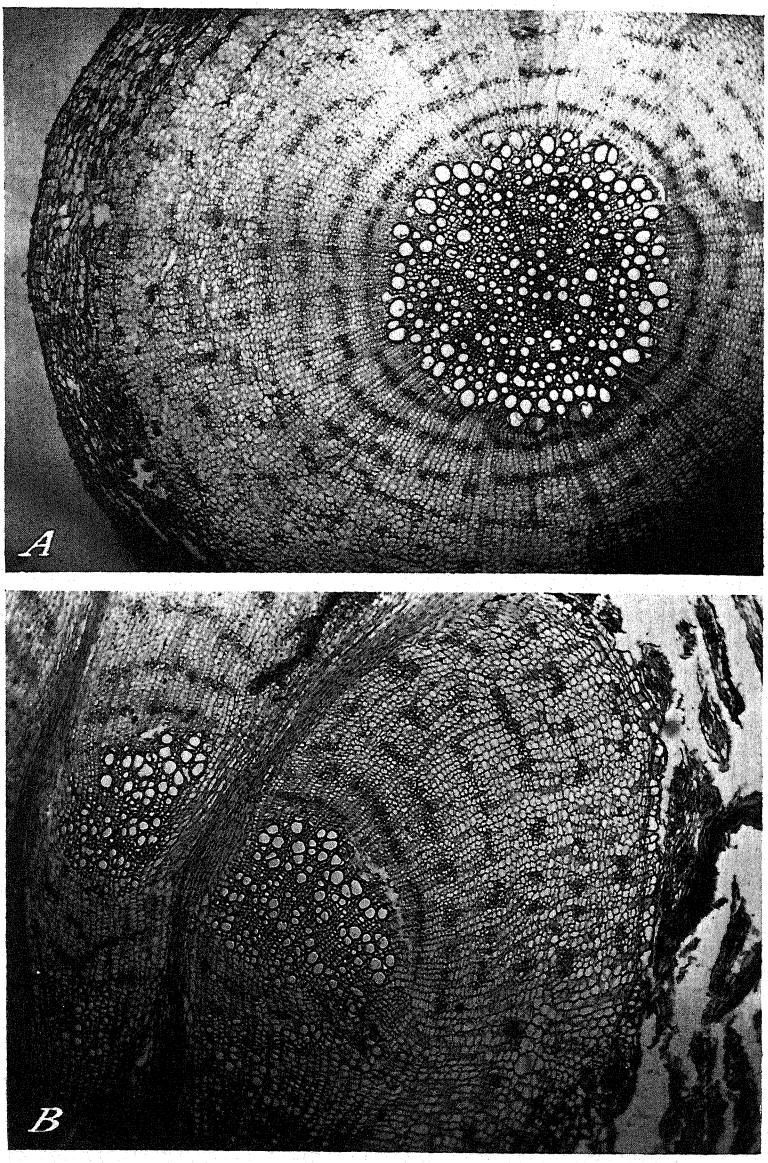


FIGURE 1.—Cross sections of dandelion root: *A*, Young root showing abundant parenchyma and scattered groups of sieve tubes in the phloem; *B*, older root showing sloughing of primary phloem and formation of interxylem phloem strands.

proliferation of the xylem parenchyma with the separation of xylem strands, the development of internal phloem, and frequently the separation of the older root into several strands, separate at the top with separate crowns but joined at the base.

RESERVES OF THE ROOT

Perennial composites frequently form inulin or levulins as storage polysaccharides. The artichoke (*Helianthus tuberosus*) and dahlia

(*Dahlia* spp.) are familiar examples. Preliminary tests showed that the dandelion root contains levulins, substances comparable to the dextrins but more easily hydrolyzed and yielding fructose instead of glucose. Levulins are distinguished from inulin by their greater solubility in cold water.

A 5-g sample of root residue, which had been killed and thoroughly extracted with 80-percent alcohol to remove sugars, dried, and ground to 200 mesh in a ball mill, was extracted 10 times with cold water. Each extraction was hydrolyzed separately with 1+100 [3] HCl at 70° C. for 35 minutes, and the reducing value of one-fifth aliquots determined by the Munson-Walker-Bertrand method as given by Loomis and Shull (*17*). The residue from the cold water extraction was then extracted 10 times with boiling water by suspending in hot water in centrifuge tubes immersed in a boiling water bath, and stirring intermittently for 30 minutes. The residue was centrifuged out and each extraction hydrolyzed separately as above and tested for reducing substances.

TABLE 1.—*Reducing substances yielded by successive hot- and cold-water extractions of a sugar-free dandelion root powder*

Extraction No.	Cold-Water extractions		Hot-water extractions		Extraction No.	Cold-water extractions		Hot-water extractions	
	Reducing substances	Percentage of total	Reducing substances	Percentage of total		Reducing substances	Percentage of total	Reducing substances	Percentage of total
	Milligram	*Percent*	*Milligram*	*Percent*		*Milligram*	*Percent*	*Milligram*	*Percent*
1	50.2	73.4	0.9	30	7	0.3	0.4	0.3	10
2	12.1	17.7	.3	10	8	.3	.4	.3	10
3	3.1	4.6	.3	10	9	.5	.7	.5	17
4	1.0	1.6	.4	13	10	.0	.0	.0	0
5	.7	1.0	.0	0					
6	.2	.2	.0	0	Total	68.4	100.0	3.0	100

The results of the two tests, given in table 1, show that only cold-water-soluble polysaccharide reserves were present. Inulin and portions of any starch present would have been extracted by the hot water, but the data indicate that only traces of material, such as would have been obtained from slow hydrolysis of hemicelluloses, were found in the hot-water extract. The absence of starch was shown by the iodine test also, and it is inferred that starch and inulin are not normal storage forms in dandelion.

The cold-water extract from 5 g of powder was made to 1 liter and 50-ml aliquots used for hydrolysis tests as indicators of the contained materials. Fifty milliliters of this extract, cleared with neutral lead acetate and hydrolyzed under inulin hydrolysis conditions (1+100 HCl at 70° C. for 35 minutes (*13*)), gave a reducing value of 17.6 mg when calculated as invert sugar. The uncleared solution gave a value of 19.4 mg which is higher than the value for the cleared solution by seven times the error of determination. The loss could have been due either to occlusion of levulins with the lead precipitate or to the presence of a levulin gum containing an acid radical and thus forming a lead salt. When the hydrolysis time at 70° C. with 1+100 HCl

[3] One part of concentrated c. p. HCl and 100 parts of water by volume. Approximately 0.43 percent hydrogen chloride by weight.

was increased by intervals from 0 to 120 minutes, the invert sugar yields from 50-ml aliquots of the solution varied as indicated below.

Period of hydrolysis at 70° C. (minutes)	*Reducing substances from 50 ml extract as invert sugar (milligrams)*
0	1. 4
10	18. 7
20	19. 2
30	19. 6
40	19. 7
60	20. 3
120	21. 3

Levulin hydrolysis should be completed in 35 minutes; it will be noticed that it was very nearly completed in 10 minutes, but that there was a slight increase in reducing value with continued heating in spite of the slow destruction of levulose by the heating. The continued rise with time suggested a mixture of dextrin and levulin, and hydrolysis with 1+20 HCl at 120° C. for 30 minutes gave a reducing sugar value of 28.7 mg.

The material was tested further for the presence of dextrins by a series of hydrolyses, with another sample of extract, in which acid concentration, heating time, and heating temperature were varied independently. The results, given in table 2, indicate the presence of a mixture of levulin and dextrin in approximately equal quantities. The reducing value of this extract after levulin hydrolysis (1+100 HCl at 70° C. for 35 minutes) was 11.6 mg invert sugar in 50 ml of extract; after dextrin hydrolysis (1+20 HCl at 120° for 60 minutes), it was 19.8 mg; and after a shorter hydrolysis at the higher temperature, to hydrolyze most of the dextrin with a minimum destruction of levulose, the value was 21.5 mg. Heating in 1+40 HCl for 120 minutes did not give a significantly different value. Shorter heating with the weaker acid gave low values, suggesting an incomplete hydrolysis of dextrins.

TABLE 2.—*Hydrolysis of cold-water extract of dandelion residue with 1+20 and 1+40 HCl at 70° and 120° C.*

Heating time (minutes)	Temperature	Reducing sugar[1] per 50 ml when hydrolyzed with indicated concentration of acid		Heating time (minutes)	Temperature	Reducing sugar[1] per 50 ml when hydrolyzed with indicated concentration of acid	
		1+20 HCl	1+40 HCl			1+20 HCl	1+40 HCl
	°C.	*Milligrams*	*Milligrams*		°C.	*Milligrams*	*Milligrams*
35	70	12.3	12.2	60	120	19.8	17.1
30	120	21.5	16.7	120	120	18.5	22.3

[1] 1+100 HCl at 70° C. for 35 minutes gave an invert sugar value of 11.6 mg per 50 ml of extract.

SEASONAL FLUCTUATIONS IN THE RESERVES OF THE ROOT

The effectiveness of any method of dandelion control which kills or removes only the upper portions of the plant will depend directly upon the food reserve level of the roots at the time of treatment. The effectiveness of other types of treatment also may be correlated with reserve level.

Samples were taken at approximately monthly intervals during the summer of 1935. The season was normal, and dandelions were

dominant in the area from which the samples were dug. The washed roots were sliced into boiling alcohol and extracted and the several fractions determined with methods given by Loomis and Shull (*17*). The reserve polysaccharide determination was simplified by hydrolyzing with 1+20 HCl for 30 minutes at 120° C. Results so obtained are approximately 10 percent low because of the destruction of levulose at the high temperature. The analytical data are summarized in table 3. The low point in total nitrogen came at the time of fruiting, but the low point in carbohydrate reserves was apparently reached during early vegetative growth. Instead of a drop during flowering, the polysaccharides, which were the dominant carbohydrate reserve, increased from the first sampling on May 1 to a high during a short dry period in July and remained high except for a dip with new growth following late July rains. The carbohydrate analyses suggest that treatments, such as cutting or iron sprays, the effectiveness of which depends upon low reserves, should be made early in the season.

TABLE 3.—*Carbohydrate and nitrogen content on fresh-weight basis of dandelion roots at different times of the year*

Date of sampling	Reducing sugar	Sucrose	Polysaccharides	Total carbohydrate	Soluble nitrogen	Protein nitrogen	Total nitrogen
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
May 1 (budding)	0.25	0.77	1.25	2.27	0.12	0.12	0.24
May 20 (bloom)	.13	.69	2.66	3.48	.04	.23	.27
June 1 (fruiting)	.33	1.09	3.38	4.80	.04	.11	.15
July 8	.56	1.08	5.53	7.17	.04	.13	.17
Aug. 6	.57	.85	2.59	4.01	.06	.17	.23
Sept. 10	.36	.74	3.68	4.78	.10	.15	.25
Oct. 16	.32	1.83	3.04	5.19	.14	.25	.39
Nov. 8	.25	1.51	3.90	5.66	.27	.20	.47

HERBICIDES FOR THE CONTROL OF DANDELION

Iron sulphate (ferrous) was widely advocated for weed control by Bolley (*5*) at the beginning of the century. He stated that dandelion plants were prevented from seeding but not killed by four sprays. Adams (*2*) and Moore and Stone (*19*) likewise recommended iron sulphate, but apparently they took their records shortly after spraying and before the plants had resprouted from the roots. Arthur (*4*), French (*7*), Fyles (*8*), and Olive (*21*) report that the leaves were quickly blackened and killed, but that the plants resprouted from the roots even after repeated treatments. French sprayed 12 times in 2 years without killing the stronger dandelion plants. On the other hand, Munn (*20*) and Longyear (*15*) reported satisfactory control with three or four sprays properly applied. In the present writer's work, single sprays in connection with heavy fertilization and under favorable conditions have given a 40-percent control, and three sprays under less favorable conditions have given no control.

Other herbicides recommended by various authors include sulphuric acid (*1*), ammonium thiocyanate,[4] furfural emulsions (*3*), ammonium sulphate (*6*), gasoline, and kerosene. Of these, the first three burn or kill the grass when applied at rates which are effective against

[4] HARVEY, R. B. AMMONIUM SULFOCYANATE AS A WEED SPRAY. Minn. Univ., Div. Plant Physiol. Mimeographed Pub. 1930.

dandelions. Gasoline burns the grass and is too volatile to be used as a spray in warm weather. Kerosene was used by Longyear (*15*) and Olive (*21*) for individual plant applications made with an oilcan, but its use as a differential spray was first reported by Loomis and Noecker (*16*).

Kerosene and other distillates have been used on the Iowa State College campus on some 200 experimental plots and on two 5-acre and one 30-acre area. The results have varied from a 50-percent mortality of dandelions and serious injury to the grass to a 100-percent mortality of dandelions from a single application and no permanent injury to bluegrass or white clover sods. Kerosene specifications and weather at and after the time of spraying appear to be the factors that determine the success or failure of the treatment.

SPECIFICATIONS OF A KEROSENE FOR LAWN SPRAYING

The boiling-point range for commercial kerosenes is commonly 180° to 250° C. Perfection kerosene, a commercial product with a boiling-point range of 185° to 240°, was compared with distillation cuts from a specially purified lot of naphtha, prepared and supplied by an oil refinery in Okmulgee, Okla. This latter product was treated to reduce the percentage of unsaturated hydrocarbons, and then fractioned into low, medium, and high boiling-point-range samples. A sample of acid-washed Red Crown gasoline having a boiling-point range of 40° to 180° was included in the test. The results of the experiment are given in table 4. They indicate that the medium boiling-point fractions were most satisfactory for the conditions of the spraying, a cool day in mid-October. The distillates having the higher boiling points left an oily residue on the sod and seriously injured or nearly killed the grass as well as the dandelions. Probably both residue and grass injury with these fractions would have been less noticeable had the spraying been done in warmer weather.

TABLE 4.—*Percentage of dandelions killed and of bluegrass injured with various hydrocarbon sprays having different boiling points, applied during cool weather in mid-October*

[Data are averages of those from three 100-square-foot plots]

Boiling-point range (° C.)	Dandelions killed	Bluegrass injury	Boiling-point range (° C.)	Dandelions killed	Bluegrass injury
	Percent	*Percent*		*Percent*	*Percent*
40–180 [1]	95	40	245–300 [3]	100	60
180–240 [2]	98	20	300–325 [3]	99	99
190–245 [3]	98	30			

[1] Acid-washed gasoline—Red Crown.
[2] Acid-washed Perfection kerosene.
[3] 3 fractions of refinery purified distillate.

Gasoline sprays in warm weather have resulted in a quick burn of both grass and dandelions, but few if any of the dandelion plants have failed to recover. Kerosene may give a poor kill, apparently because of its volatility, when used in hot weather, and it seems probable that a dandelion spray for general conditions could include distillates up to 275° C. when mixed with the lighter kerosene. Kerosene (180°–250°), however, can be used alone in any but the hottest

weather. The inclusion of fractions with a boiling-point range below 180° would be permissible in cool weather but would add to the cost of the treatment.

The unsaturated hydrocarbon content of distillates has been shown (*22*) to be a major factor in their toxicity to foliage, and the writer has found that oils containing considerable percentages of unsaturated compounds destroy bluegrass sods as well as dandelion plants. A number of distillates of the kerosene boiling-point range of 180° to 250° C. and different percentages of unsaturated hydrocarbons have been used in various tests. Perfection kerosene is described by the refiners as a straight-run distillate, that is, one obtained from the first distillation of the crude oil before cracking treatments are used, and as containing from 2.5 to 4 percent of unsaturated hydrocarbons. Recycle gas oil, also known as tractor distillate, is the same boiling-point cut on the cracked residue from the first distillation. The breaking of the long-chain, high-boiling-point hydrocarbons in cracking leaves many unsaturated compounds, and recycle gas oil may run 20 percent or more unsaturated. Distillates prepared for such products as fly-spray bases are washed with concentrated sulphuric acid or equivalent treatments which reduce the percentage of unsaturated compounds to approximately 1 percent. Whenever these three preparations have been compared it has been found, as shown in table 5, that the gas oil or tractor distillate destroys bluegrass as well as dandelions. Oil with 3 to 4 percent of unsaturated compounds appears to be safe under favorable conditions. Commercially purified oils, or kerosenes washed with concentrated sulphuric acid, have, in three trials, been somewhat less effective in eradicating dandelions than the straight-run kerosene. Injury to the grass by late fall (November 1) spraying was not prevented by washing kerosene with acid. More exact determinations of the tolerance of bluegrass to unsaturated hydrocarbons are needed, but present findings indicate that an unsaturated content of not more than 4 percent should be specified.

TABLE 5.—*The relation of unsaturated hydrocarbon content of distillate sprays to dandelion control and grass injury*

[Data are averages from three replicated plots]

Hydrocarbon used	Percent unsaturated [1]	Mature dandelions per 100 square feet	Dandelions killed	Grass injury	Seedling dandelions per 100 square feet [2]
		Number	*Percent*	*Percent*	*Number*
Check		657	0	0	23
Perfection kerosene	3	10	98.5	[3] 27	80
Acid-washed kerosene	1	10	98.5	30	80
Gas oil	25	3	99.5	100	370
Standard tractor distillate	18	3	99.5	85	120
Acid-washed distillate	3–5	3	99.5	40	30

[1] Average figures for type of material used.
[2] Seedling counts in May after spraying in October; they represent new invasion.
[3] Injury more severe than normal because sprays were applied in late October.

CONTROL OF DANDELIONS WITH KEROSENE

METHODS OF APPLICATION

Two methods of applying kerosene may be used. Individual plants may be sprayed (fig. 2, *A*) when scattered plants are to be eradicated,

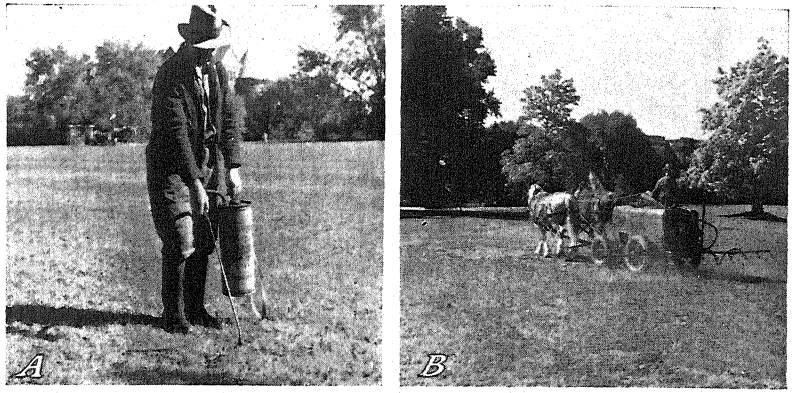


FIGURE 2.—Applying kerosene for dandelion control: *A*, Method for scattered plants or small areas; *B*, spraying large areas.

or broadcast sprays (fig. 2, *B*) may be used for larger areas and heavier infestations. Individual plant applications can be used over a wider range of conditions and are safer for lawn use. Just enough kerosene to wet the crown and most of the leaves of the plant should be applied as a fine spray. If a lawn is treated in part by spotting and in part by spraying, an irregular growth for 2 or 3 weeks will result from the checking of the growth of the grass in the sprayed areas.

Power sprayers, such as the one illustrated in figure 2, *B*, should be adjusted to cover the surface uniformly at a rate of 200 gallons an acre. Kerosene atomizes readily and a high pressure is not required in its application. Although such a machine has not been used, good results would be expected with a traction-driven potato sprayer. If the spray is applied in the fall, as recommended, incidental spraying of deciduous shrubbery will do no harm. Evergreens should not be sprayed.

WEATHER FACTORS

Kerosene may be applied to wet grass or during a rain without appreciable loss of effectiveness. In fact, sprays so applied, because of less loss from evaporation, may be more effective than those applied in clear weather. The opposite relationship holds for ferrous sulphate sprays which are washed from the leaves by rain.

Temperature and sunshine are important factors in the effectiveness of kerosene sprays for dandelions. One comparison of their effect was made on July 21, 1935. One of two plots was sprayed at 10:30 a. m. in bright sunlight, temperature 35° C.; the second plot was sprayed after sundown of the same day, temperature 22°. On October 1 the percentage mortality of dandelions was 20 and 85, whereas grass injury was 80 and 0 percent. Additional data are given in table 6.

TABLE 6.—*Relation of weather factors to the effectiveness of kerosene sprays, 1935*

[Dandelion counts on May 5, 1936; all data are average of three replications]

Spraying treatment [1]	Mature dandelions per 100 square feet	Dandelions killed	Grass cover	Seedling dandelions per 100 square feet
	Number	*Percent*	*Percent*	*Number*
Check	699		34	51
Sept. 11, 26° C., sun, 5 p. m.	213	67	43	209
Sept. 25, 22°, cloudy	15	98	18	217
Sept. 28, 18°, sun	56	92	30	330
Oct. 15, 19°, cloudy	71	90	7	500
Nov. 23, 7°, cloudy	0	100	.7	1,660

[1] All were made with Perfection kerosene at the rate of 250 gallons an acre.

The applications on September 28 in sunny weather were less effective than those made 3 days earlier on a cloudy day, but were no less effective than applications made on a cloudy day in mid-October. The results suggest that sunshine does not affect the value of the spray, at least at the lower temperatures, except as it increases the vaporization of the kerosene before it has penetrated into the roots of the dandelion plants.

In contrast to the serious grass injury observed in plots sprayed November 23 at 7° C. (table 6), no permanent injury was noted in a

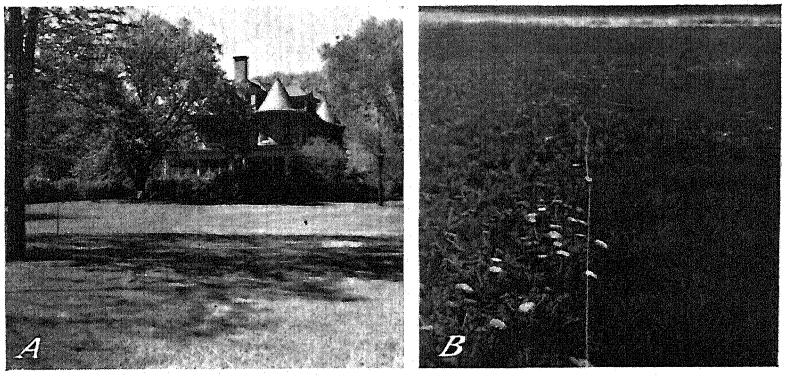


FIGURE 3.—The control of dandelions with kerosene: *A*, A 99-percent control without grass injury in a lawn sprayed October 17, 1936; *B*, persistence of control in plots at right given one spray 2 years before photograph was taken.

series of 32 plots sprayed at the same temperature on September 30, 1934, and the degree of dandelion control was the same (99 to 100 percent) in both tests. All the observations on temperature may be summarized by saying that good results were obtained at temperatures up to 23° by spraying in the evening or on cloudy days. The usual result of higher temperature has been reduced effectiveness, and occasionally greater grass injury as well. Temperatures down to freezing have not prevented good results when other factors were favorable. In a large area sprayed October 17, 1936, with a power sprayer, no grass injury was observed the following June on exposed locations (fig. 3, *A*), but injury amounting to complete kill was noted on steep north slopes of the same area and in the shade of evergreen trees. Dandelion control was better than 98 percent throughout the

plot. These results, in connection with those given above, suggest that a certain amount of warm weather after spraying reduces the kerosene residue in the sod by evaporation, and prevents permanent injury. A higher temperature, particularly at the time of spraying and in combination with bright sunshine, causes excessive evaporation and reduces the quantity of kerosene reaching the dandelion roots to less than the lethal dose.

Seasonal Factors

The larger differences in temperature have been obtained, in most cases, by varying the season of application. Kerosene applications have been made at frequent intervals between May 1 and December 1 with good control in at least some of the plots. Early spring treatments (as soon as the dandelions are well up) have given good control of old plants and satisfactory final results on plots with a good stand of grass. When the dandelions were thick and the grass thin, too much bare area was left on which dandelion seedlings became established during late spring. As tables 5 and 6 show, treatments that injured the grass were followed by high seedling counts.

Midsummer applications of kerosene are difficult to make on a large scale because the work must be done in cloudy weather. Late fall applications have resulted in serious winter injury to the grass (table 6). Early fall applications (September 15 to October 10 at Ames) have given the best results. Two factors appear to be involved—the more generally moderate temperatures, and the double growing season, both fall and spring, for the closing of the bared areas by stolon growth of the grass. Sprayed bluegrass makes no growth for 2 to 3 weeks after the treatment. Fall applications should allow for 4 to 6 weeks of normal growth after this temporary set-back; that is, sprays should be made 6 weeks or 2 months before the expected date of grass-killing freezes or deep snow.

The Physiological Action of Kerosene

Dandelions sprayed with kerosene develop a dark-green color within a few seconds as the oil penetrates the intercellular spaces of the leaves. The dark color disappears within 24 to 36 hours, and for the next 6 to 20 days the plants appear normal to yellowish. The odor of kerosene usually can be detected on root fragments during this period; the phloem becomes translucent and the quantity of latex exuded from a cut root surface increases. The first conspicuous symptom of injury is a reddening or bronzing of the leaves which appears about 1 week after spraying in warm weather and 2 to 3 weeks after spraying in cooler weather. This change in color was accompanied, as shown by the data in table 7, by a fourteenfold increase of sucrose in the leaves. Polysaccharides (levulins plus dextrin), reducing sugars, and soluble nitrogen showed moderate increases in the leaves of the sprayed plants in the 2 weeks after treatment. Changes in the root consisted principally of a digestion of polysaccharide to reducing sugars with less change in sucrose and total carbohydrates. The slow digestion of the reserves of the root and the rapid accumulation of carbohydrates, particularly sucrose, in the leaves may be considered as indications of interference with translocation. This conclusion was supported by histological studies which showed necrosis of the phloem in the roots of the sprayed plants and the accumulation of

TABLE 7.—*The chemical composition on May 20 and June 1 of the leaves and roots of nonsprayed dandelion and of dandelion sprayed with kerosene on May 18, 1935*

Date of sampling and fraction	Leaves		Roots	
	Control	Sprayed	Control	Sprayed
May 20, 1935:	*Percent*	*Percent*	*Percent*	*Percent*
Reducing sugars	0.40	0.28	0.13	0.17
Sucrose	.23	.45	.69	.35
Polysaccharides	1.01	1.06	2.66	2.87
Soluble nitrogen	.03	.03	.04	.04
Total nitrogen	.31	.27	.27	.21
June 1, 1935:				
Reducing sugars	.24	.64	.33	1.03
Sucrose	.16	2.43	1.09	1.04
Polysaccharides	1.16	1.58	3.38	2.71
Soluble nitrogen	.02	.03	.04	.05
Total nitrogen	.18	.16	.15	.17

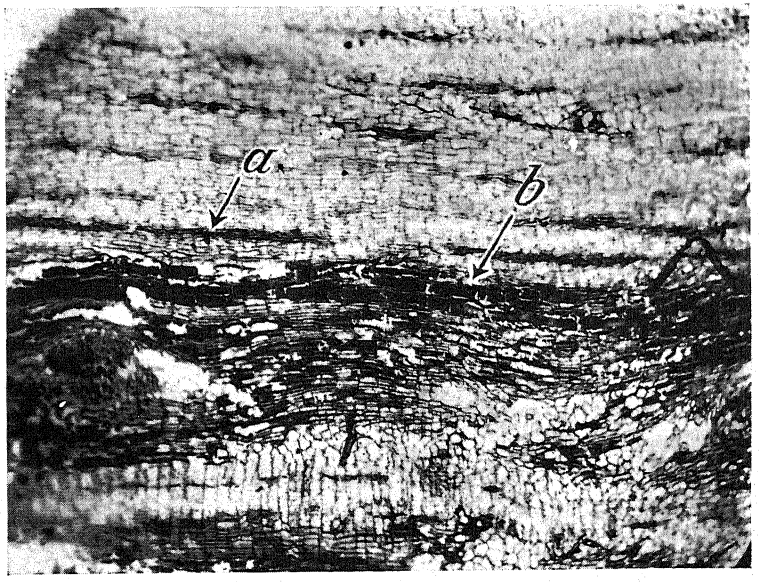


FIGURE 4.—Kerosene injury in dandelion root: Phloem necrosis at *a*, and plugging of outer xylem with latex at *b*.

latex (fig. 4) in the outer vessels. Injury was commonly observable to depths of 30 cm, and the death of the tops appeared to be due to the previous death of the roots.

Bluegrass (both *Poa pratensis* and *P. compressa*), white clover (*Trifolium repens*), common plantain (*Plantago major*), and buckhorn (*P. lanceolata*) have not been seriously injured by kerosene sprays applied under the conditions recommended. Foxtail (*Setaria* spp.) and crabgrass (*Digitaria* spp.) have shown more injury than bluegrass, but have survived in some treatments. The action of kerosene is thus seen to be rather specific for dandelion. Possibly the leafy rosette and fleshy taproot of this plant are favorable to the absorption and retention of the oil. Even under favorable conditions, bluegrass may be killed by appreciably heavier applications than the 200 gallons an

acre recommended, or under conditions unusually favorable to the persistence of the oil within the bluegrass plants, and a greater accumulation and longer retention of the kerosene in the dandelion root would appear to be the most probable explanation of the greater toxicity of the light applications on this plant. A second factor in differential killing is the ability of isolated bluegrass buds to produce roots and grow, even when much of the rhizome system has been destroyed, whereas dandelion plants do not recover after the taproot is killed.

THE CARE OF TREATED LAWNS

The heavy seeding and ready distribution of the dandelion fruit insure the presence of the plant in any available area. However, the seedlings do not compete with well-established sod, particularly in moderately dry weather. Figure 3, *B*, shows a Canada bluegrass (*Poa compressa*) sod which was still practically free of dandelions 2 years after it had received a single kerosene spray. This plot was fertilized twice during this interval and observations showed that the many dandelion seedlings which had started on the area each spring died during July and August before their root systems had penetrated below the level of the grass roots.

The sensitivity of dandelion seedlings to dry weather is shown by the behavior of the seedlings on the plots sprayed November 23, 1935, which were estimated to have an average of 1,660 seedlings per 100 square feet the following May (table 6). Less than 10 dandelions per plot, or about 0.5 percent, of these seedlings survived the drought of 1936. Older, deeper rooted plants are seldom injured by dry weather and more than 80 percent of the large plants on the check plots survived the 1936 drought.

Injudicious sprinkling during the summer months will aid in keeping dandelion seedlings alive and will hasten reinfestation. Ammonium sulphate fertilization (*12*) and ferrous sulphate sprays, heavy but infrequent watering, and less mowing during dry weather with the mower set high at all times, will retard reinfestation. Some spotting work with a kerosene spray will be required at intervals to remove plants which become established in breaks in the sod.

Especial care is required to insure the rapid resodding of lawns in which large numbers of dandelions have been killed and much of the ground left bare. Reseeding is rarely successful because dandelion and grass seedlings develop under similar conditions. Natural spread of the grass by stolons must, therefore, be depended upon. Frequently a year of treatment with ammonia fertilization and iron sulphate spraying should precede the kerosene to encourage the establishment of grass between the dandelion plants. With a month of cool moist weather, good sods may be reestablished, if there is grass among the dandelions, by applying ammonium sulphate at the rate of 150 to 300 pounds per acre and phosphorus and potassium as needed. Good sod has been established without reseeding in a single season from scattered bluegrass plants which covered less than 1 percent of the area in the spring.

One factor in the handling of sprayed lawns that needs further investigation is the matter of early spring mowing. The recent emphasis on the importance of reserves in grasses (*9*, *11*) has led to the belief that spring cutting or pasturing of bluegrass should be delayed.

Graber (*10*) has shown, however, that close cutting of fertilized sod allowed to grow tall may seriously check the development of the grass, and Leukel and Coleman (*14*) have shown that Bahia grass allowed to grow naturally and to produce seed made a much poorer sod than grass cut at intervals. A successful lawn cultural practice should maintain a neat appearance, and moderately close mowing at some stage is necessary. It seems probable that spring mowings should be made early enough and frequently enough to prevent seed formation or the shading out of the lower leaves of the grass. If the mower is set to cut at a height of 1½ to 2 inches, frequent cutting will encourage the development of leaves near the ground and will permit full leaf coverage for photosynthesis while keeping the lawn neat and smooth.

In contrast to this spring treatment, the lawn should be mowed infrequently or not at all during dry weather, and mowing should stop a month before the end of the growing season in the fall to encourage the accumulation of reserves for winter.

SUMMARY

The food reserves of the common dandelion (*Taraxacum officinale*) are shown to consist largely of a mixture of levulin and dextrin, and to have reached a low level for the samples tested during early spring growth.

Straight-run kerosene with a boiling-point range of 180° to 250° C. and an unsaturated hydrocarbon content of not over 4 percent has been shown to be a very effective differential spray for dandelions in bluegrass-white clover lawns. The undiluted kerosene is sprayed uniformly over the lawn during cool, cloudy weather, at the rate of 200 gallons an acre. The best results have been obtained by spraying 2 months before the end of the bluegrass growing season, or September 15 to 30 at Ames, Iowa. The kerosene appears to accumulate and to be retained in the taproot of the dandelion and to affect the plant through the destruction of the phloem of the root. Other common lawn plants and weeds show little or no injury from applications of kerosene which are effective against dandelion. Scattered dandelion plants may be eradicated at any time by spraying the individual plants lightly with kerosene.

Bluegrass as well as dandelions will be killed (1) if more than about 300 gallons of kerosene are used instead of the recommended 200; (2) if furnace or tractor distillates are used instead of kerosene; and (3) if the spray is applied too late in the fall to permit the grass to make a partial recovery before winter.

The rapid reestablishment and maintenance of a close sod with the avoidance of frequent, light waterings during dry periods are important in keeping dandelions from becoming established in lawns. The dandelion seedlings will not survive a dry period of moderate intensity when growing in heavy sod, but they may become established in open areas or if sprinkled frequently. Older plants with roots below the level of the grass roots should be eradicated with kerosene.

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INFLUENCE OF CERTAIN ENVIRONMENTAL CONDITIONS ON CONGESTION OF STARCH IN TOMATO PLANT STEMS [1]

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INTRODUCTION

The phenomena of photosynthesis, the synthesis of the simpler carbohydrates, the translocation of these materials to all parts of the plant, and their further transformation into starch to be stored have been subjects of speculation and experimentation for many years. Since starch is one of the most widely distributed plant products, investigators have been considerably interested in determining the relation of various factors, such as mineral elements and environmental conditions, to the accumulation or congestion of starch in various parts of the plant. The relation of potash to the formation of starch in plants and its subsequent hydrolysis when needed for further growth has received considerable attention, but specific tangible results appear to have been difficult to obtain. The part that potash plays in the formation and utilization of starch is uncertain, but it presumably acts as a catalyst and has radioactive properties (*4*).[2] An enormous amount of data has been presented in the literature which demonstrates clearly that nitrogen deficiency plays a very definite part in the accumulation of starch or other carbohydrates in the plant. High nitrogen content is generally accompanied by medium to low carbohydrate content, and very low nitrogen usually indicates high carbohydrate accumulation.

A number of investigators have demonstrated rather definitely that starch accumulation, or congestion, takes place in tomato plants under conditions of deficient nutrient supply or under environmental conditions that are accompanied by retardation of growth. However, there appear to be very few quantitative data in the literature showing the interaction of fertilizer deficiency or nitrogen deficiency and other unfavorable environmental conditions, such as soil moisture and temperature, in relation to the congestion of starch in the tomato plant.

The present paper presents data showing the influence of fertilizers, soil moisture, temperature and humidity, and evaporating power of the air on the accumulation of starch in the stems of tomato plants (*Lycopersicon esculentum* Mill.). The data were secured incidentally in a study of the effect of environmental conditions on the growth responses of tomatoes as related to the appearance of certain nonparasitic diseases and other abnormal physiological disturbances.

REVIEW OF LITERATURE

There is an extensive literature that relates to the various factors influencing the accumulation of the simpler carbohydrates and the

[1] Received for publication October 1, 1937; issued June 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 881.

total hydrolyzable polysaccharides, but that relating to congestion of starch as such is not extensive. Hartwell (*2*) appears to have first recognized the effects of unfavorable conditions on the congestion of starch in potatoes and beets. At first he was of the opinion that starch congestion was due mainly to potash deficiency, but later he came to the conclusion that any condition unfavorable to plant growth would produce the phenomenon. Unfortunately, Hartwell did not secure quantitative data, but instead he made a microscopic estimation of the starch present. Kraus and Kraybill (*5*), in their work on vegetation and reproduction, presented extensive data to show that starch accumulates in tomato stems when the plants are grown with a deficient nitrogen supply. Jansen and Bartholomew (*3*) found that the amount of potassium present in the plant was correlated with the total sugars, but they seemed to be doubtful as to its relation to starch accumulation or congestion. In addition to the above citations, a large amount of work has been recorded in the literature showing the effects of low temperature on the accumulation of polysaccharides, on the synthesis of nitrogen compounds, and on general metabolism in the plant. The work of Nightingale (*6*) on the effects of temperature on metabolism and accumulation of carbohydrates in tomatoes demonstrated the responses of plants to different temperatures, but he, as well as others, apparently did not consider it necessary to maintain accurately controlled soil moisture. There seems to have been a rather general lack of appreciation of the very marked differences in growth and physiological behavior that result from relatively small differences in water supply.

METHODS AND MATERIALS

Experiments were conducted at the Arlington Experiment Farm, Arlington, Va. The methods of procedure were rather unique. The tomato plants were grown in a good quality of greenhouse soil made up of loam from the Arlington farm, composted with manure and muck, to which sand was later added. All soil before being used was passed through a ¼-inch-mesh wire screen to insure uniformity. This soil had a water-retaining capacity of 65 percent of its dry weight, and the water-retaining capacity remained uniform over a period of 5 years of experimental work.

The plants were grown at 3 soil-moisture levels of approximately 38, 47, and 56 percent of the dry weight of the soil. On a water-retaining-capacity basis, these were, respectively, 58, 72, and 86 percent. These series were set up by first determining the original amount of water in the soil and then adding sufficient water to bring the water content up to the required percentage. The soil moisture was maintained at approximately the desired level by frequent additions (four to six times daily) of measured amounts of water, the required amounts being determined by weighing the cultures on solution balances of 40-kg capacity.

All plants were grown in 3-gallon glazed crocks holding 12 kg of soil. As the soil was placed in the crocks, fertilizer containing various proportions of nitrate of soda, superphosphate, and potassium sulphate was mixed with it at the rate of 2 tons per acre. Three fertilizer formulas were used, namely, 12–0–12, 12–6–12, and 6–12–6, in the

order of nitrogen, phosphorus, and potassium, respectively; the checks received no fertilizer treatment.

Five crocks of each fertilizer treatment were placed in each of the three soil-moisture series, and these were replicated in each of three different temperature units of the greenhouse. This gave for study 5 replications at each of the 36 points of observation and a total of 180 plants under almost perfectly controlled environmental conditions.

The seeds (Marglobe variety), which were first sown in flats August 15, 1934, were transplanted to 2½-inch pots on August 25 and to the 3-gallon crocks on September 4, where they were allowed to become established. The plants were staked with heavy wire and later, with the appearance of the first flower cluster, the wire stakes were replaced

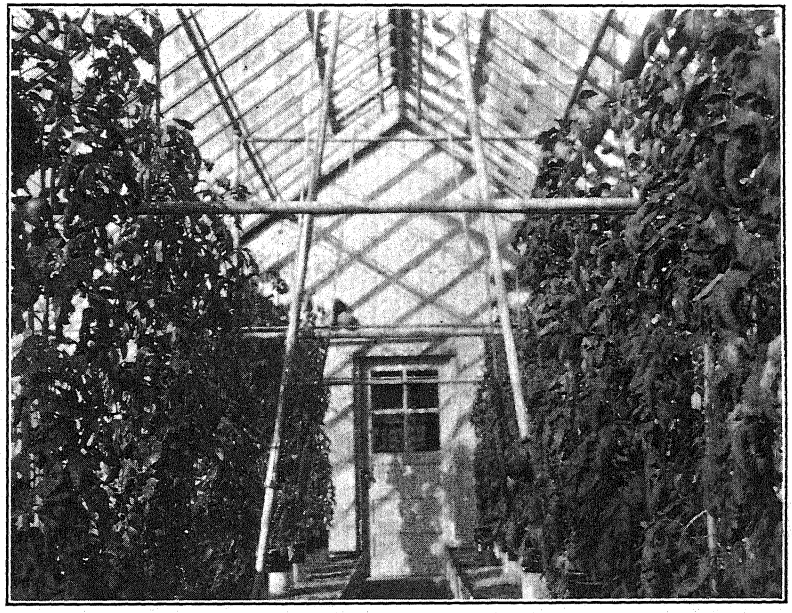


FIGURE 1.—High-temperature greenhouse, unit 1, with daily mean temperature of 74° F. Note tall spindling growth habit of tomato plants, due mainly to high soil moisture and high temperatures.

with 6-foot bamboo stakes. The plants were pruned to a single stem, tied to the stakes, and eventually topped after the appearance of the sixth flower cluster. During the growth period, the moisture content of the soil was kept approximately constant by the method already mentioned. A careful record was kept of the amount of water consumed by each plant daily for the duration of the experiment.

GROWTH HABIT AND RESPONSE OF TOMATO PLANTS TO ENVIRONMENT

The influence of temperature on the growth response and habit of tomato plants is shown in figures 1, 2, 3, and 4, which are from photographs of the mature plants grown in the three different greenhouse temperature units. In the high-temperature unit (fig. 1) the plants

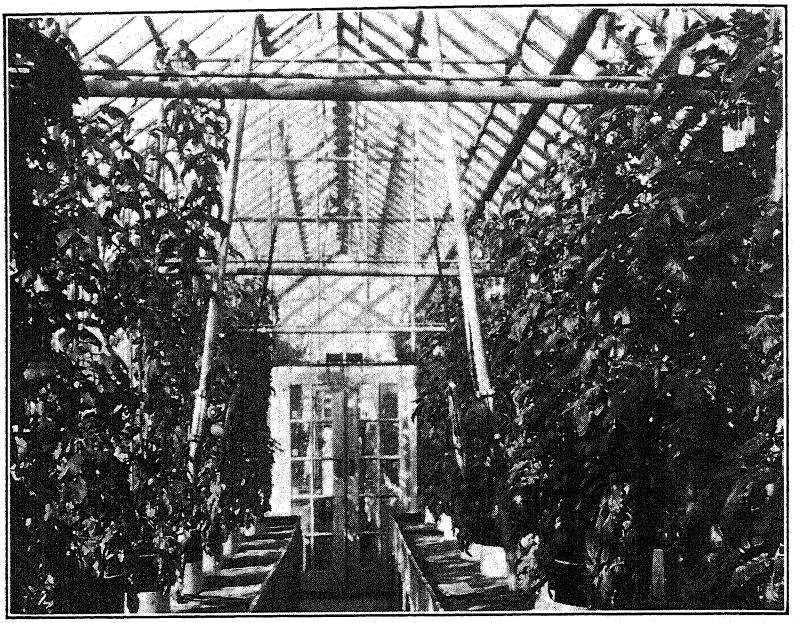


FIGURE 2.—Medium-temperature greenhouse, unit 2, with daily mean temperature of 70° F. Tomato plants not so tall as at 74°, but stockier, with thicker leaf growth.



FIGURE 3.—Low-temperature greenhouse, unit 3, with daily mean temperature of 65° F. Note short, stocky growth habit of tomato plants, with heavy stems and much heavier leaves than those shown in figures 1 and 2.

were tall and slender with relatively small stems and long internodes; they were practically barren, succulent, and of the spindling

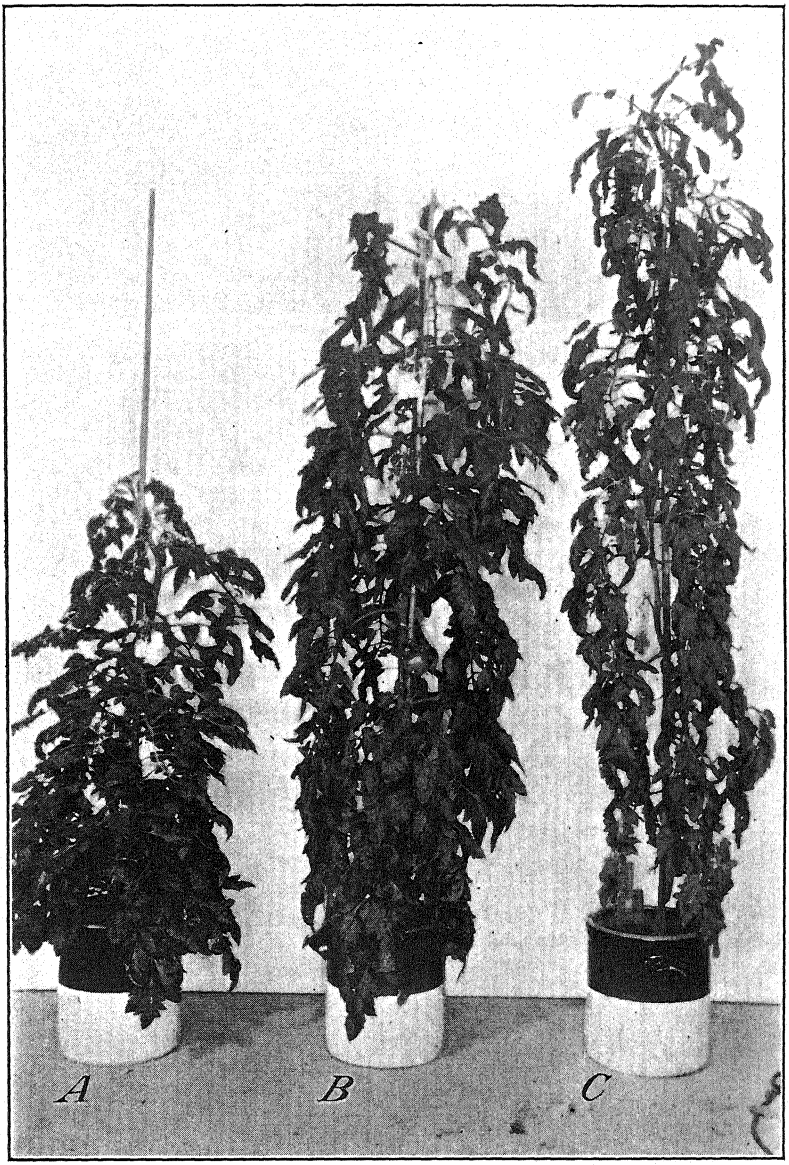


FIGURE 4.—Tomato plants showing influence of different temperatures on growth. Cultures grown at (*A*) 65°, (*B*) 70°, and (*C*) 74° F. All grown at 86 percent of soil-water-retaining capacity and with 12–6–12 fertilizer.

type (fig. 4, *C*). In the medium-temperature unit (fig. 2) the plants were shorter, stems were heavier, and leaves were spaced closer together. The plants had better color and vigor than those in the

higher temperature unit, and they were quite fruitful (fig. 4, *B*). In the lowest temperature unit (fig. 3) the plants were considerably shorter, had dense green foliage (fig. 4, *A*), and the appearance of being very succulent, although they contained much larger percentages of dry material than those of the other units. In figure 5 is illustrated the influence of soil moisture on plant growth and habit when other conditions are uniform. These plants were grown in the 1932 series of experiments, at 76° F. mean temperature, and

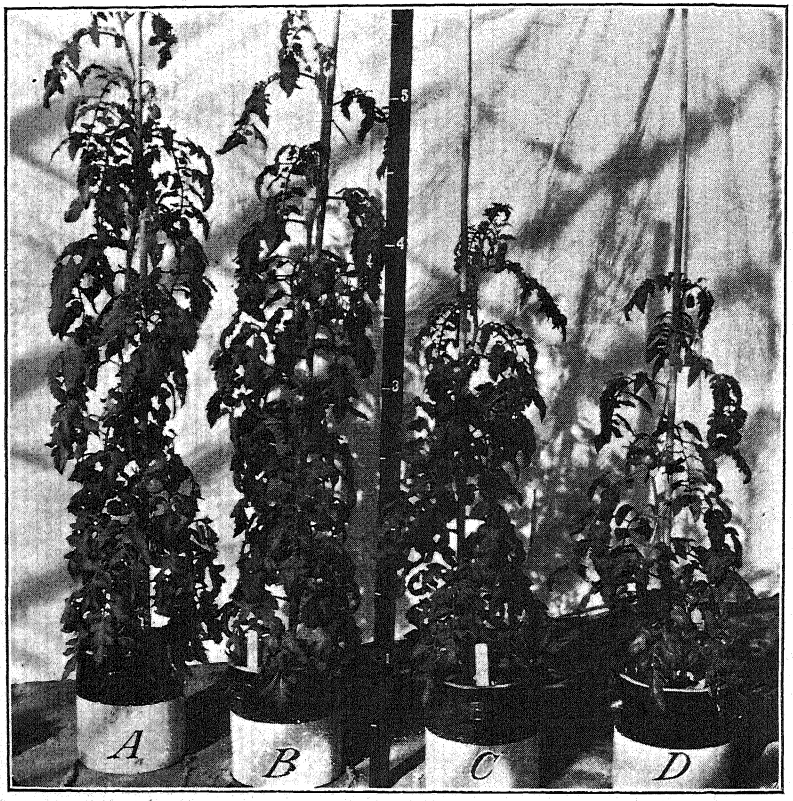


FIGURE 5.—Tomato plants showing influence of soil moisture on growth. Cultures grown at (*A*) 65, (*B*) 56, (*C*) 47, and (*D*) 39 percent of soil-water-retaining capacity. All grown at 76° F. and with 12–2–2 fertilizer. Plants from a series grown in 1932.

treated with a 12–2–2 fertilizer. Figure 4 shows the very marked influence of temperature on growth and habit of the tomato plants, all other conditions being uniform, with soil moisture at 86 percent of water-retaining capacity, and 12–6–12 fertilizer.

It was intended to operate the greenhouse units at 75°, 70°, and 65° F., respectively, from the beginning of the experiment, September 10, but high outside temperatures made it impossible to keep the units at these temperatures until about the first week in October. Table 1 shows the weekly mean temperatures for the three units as calculated by planimeter readings from hygrothermograph charts.

For the duration of the experiment the mean temperatures were 74.3°, 70.4°, and 65.7° in units 1, 2, and 3, respectively. From October 2 to December 17, during which period quite constant temperature differences could be maintained, the means were 74.6°, 69.1°, and 62.9°, respectively.

The mean relative humidities of units 1, 2, and 3 for the entire period were 55.3, 60.0, and 63.1 percent, respectively. This order of difference existed throughout the period regardless of the temperature, probably as a result of differences in ventilation incidental to attempts to regulate temperatures.

The data of the atmometric indexes of the three greenhouse units are summarized and presented in table 2.

TABLE 1.—*Mean temperatures and average relative humidities of three greenhouse units in which the cultures were grown, 1934*

Week ended	Weekly mean temperature			Relative humidity		
	Unit 1	Unit 2	Unit 3	Unit 1	Unit 2	Unit 3
	° *F.*	° *F.*	° *F.*	*Percent*	*Percent*	*Percent*
Sept. 10	73.5	74.5	74.0	61.5	67.3	69.9
Sept. 17	75.1	76.0	75.2	61.4	68.7	72.6
Sept. 24	71.1	71.2	71.4	49.6	62.8	66.2
Oct. 1	73.5	74.0	73.4	59.7	63.6	65.2
Oct. 8	68.9	66.9	65.8	56.3	60.0	62.6
Oct. 15	73.5	69.6	66.8	49.8	54.5	55.5
Oct. 22	75.1	71.5	65.0	53.9	58.0	60.6
Oct. 29	75.0	69.4	63.0	54.9	57.5	62.5
Nov. 5	74.3	69.7	62.2	54.1	58.0	60.3
Nov. 12	75.3	68.8	61.7	52.6	58.5	60. .
Nov. 19	76.3	68.8	61.6	52.4	54.5	55.8
Nov. 26	75.8	70.2	63.4	59.5	62.7	68.0
Dec. 3	76.0	69.6	62.9	64.0	69.8	75.3
Dec. 10	74.0	67.4	59.7	50.8	55.0	59.5
Dec. 17	77.1	68.1	60.1	45.9	48.7	52.4
Average	74.3	70.4	65.7	55.3	60.0	63.1

TABLE 2.—*Effects of certain environments on congestion of starch in tomato plant stems, and the relation of plant and fruit growth, water requirement, and chemical composition on a dry-weight basis for the 1934 crop*

Soil moisture (percent)	Fertilizer formula	Unit 1.—Average daily mean temperature, 74° F.; relative humidity, 55 percent; corrected evaporation from atmometers, black 19.8 ml, white 14.8 ml, difference 5 ml						
		Dry weight of plants	Weight of green fruit	Water requirement [1]	Dry material [2]	Polysaccharides	Starch	Total nitrogen
		Grams	*Grams*	*Cc*	*Percent*	*Percent*	*Percent*	*Percent*
38 [3]	12-0-12	32.9	585	579	12.09	16.06	1.54	1.22
	12-6-12	30.2	670	559	12.74	15.70	1.68	1.27
	6-12-6	44.1	910	572	11.98	16.70	1.59	1.25
	Check	46.0	790	668	11.36	14.53	1.27	.99
47 [4]	12-0-12	63.0	1,180	629	11.11	14.30	.62	1.25
	12-6-12	64.3	735	620	9.22	14.53	1.21	1.74
	6-12-6	92.5	1,745	678	12.47	14.68	1.58	.50
	Check	84.0	1,400	721	14.63	21.88	9.40	.44
56 [5]	12-0-12	118.3	1,585	694	10.37	14.38	.46	1.18
	12-6-12	92.9	2,145	685	10.49	15.08	1.33	.97
	6-12-6	99.2	2,120	721	8.56	18.80	1.90	.49
	Check	94.9	1,385	786	12.66	17.62	3.87	.23

See footnotes at end of table.

TABLE 2.—*Effects of certain environments on congestion of starch in tomato plant stems, and the relation of plant and fruit growth, water requirement, and chemical composition on a dry-weight basis for the 1934 crop*—Continued

Soil moisture (percent)	Fertilizer formula	Unit 2.—Average daily mean temperature, 70° F.; relative humidity, 60 percent; corrected evaporation from atmometers, black 16.5 ml, white 14.0 ml, difference 2.5 ml						
		Dry weight of plants	Weight of green fruit	Water requirement [1]	Dry material [2]	Polysaccharides	Starch	Total nitrogen
		Grams	*Grams*	*Cc*	*Percent*	*Percent*	*Percent*	*Percent*
38 [3]	12-0-12	34.6	700	450	13.58	15.99	2.90	1.24
	12-6-12	24.9	480	398	12.24	16.18	1.94	1.38
	6-12-6	32.6	840	491	12.33	15.98	2.42	1.27
	Check	69.1	910	632	16.69	23.68	11.56	.63
47 [4]	12-0-12	68.2	1,215	493	10.13	14.98	1.67	1.39
	12-6-12	72.0	1,320	472	10.35	14.50	1.92	1.38
	6-12-6	81.6	1,555	535	11.66	14.60	1.65	.91
	Check	97.3	1,175	648	15.51	23.98	12.70	.22
56 [5]	12-0-12	98.9	1,555	558	8.64	14.70	.58	1.41
	12-6-12	87.1	1,515	547	9.01	14.76	2.19	1.28
	6-12-6	92.8	1,840	618	10.86	14.66	1.82	.70
	Check	96.7	1,255	721	13.00	18.91	6.14	.59

Soil moisture (percent)	Fertilizer formula	Unit 3.—Average daily mean temperature, 65° F.; relative humidity, 63 percent; corrected evaporation from atmometers, black 15.1 ml, white 12.7 ml, difference 2.4 ml						
		Dry weight of plants	Weight of green fruit	Water requirement [1]	Dry material [2]	Polysaccharides	Starch	Total nitrogen
		Grams	*Grams*	*Cc*	*Percent*	*Percent*	*Percent*	*Percent*
38 [3]	12-0-12	35.6	225	317	12.41	20.62	8.49	1.35
	12-6-12	54.3	150	302	12.26	19.18	5.61	1.42
	6-12-6	68.1	650	446	12.45	19.50	5.55	.91
	Check	84.8	910	535	20.36	33.65	22.90	.21
47 [4]	12-0-12	78.2	850	409	10.66	14.35	3.11	1.34
	12-6-12	84.5	845	336	9.78	14.92	2.39	1.54
	6-12-6	113.5	655	452	10.02	14.98	3.64	1.23
	Check	97.4	1,085	551	17.95	35.17	24.10	.36
56 [5]	12-0-12	116.3	1,110	445	8.69	11.86	1.09	1.70
	12-6-12	73.1	325	438	7.53	13.28	2.32	2.22
	6-12-6	121.0	1,455	486	10.97	18.51	6.32	.46
	Check	106.8	1,345	590	17.35	31.10	19.52	.31

[1] Per gram dry weight of tops.
[2] Percentage of fresh weight.
[3] 58 percent of water-retaining capacity.
[4] 72 percent of water-retaining capacity.
[5] 86 percent of water-retaining capacity.

It is important to note that in growth and general vigor the plants used in all of these experiments were similar in size and character to what might be expected under field conditions. The plant growth approached normal, and it is for this reason that the results to be presented are unique and distinct from those of similar lines of work reported by other investigators, who have used sand or solution cultures that resulted in stunted and abnormal growth.

CHEMICAL METHODS

The samples for chemical analyses of the stems were collected on December 12, 1934, from mature plants of the types illustrated in figures 1, 2, and 3. The leaves were previously removed and preserved in 75-percent alcohol, and 100-g samples of the top parts of the stems were sliced into 75-percent alcohol. To insure uniformity of samples, all collections were made early in the morning on cloudy or rainy days. In the collection, preparation, and preservation of samples in alcohol, essentially the same methods were followed as were used by Boswell (*1*). In the further preparation of the samples for analysis, Boswell's methods were also followed, except that the dry material was ground in a Wiley type laboratory mill, F. R. I. model, to pass through a 100-mesh wire screen. In making the starch deter-

minations, the method developed by Walton and Coe (7) was used. It proved to be very satisfactory for this type of material, and duplicate or triplicate determinations consistently were in good agreement.

INFLUENCE OF ENVIRONMENT ON WATER REQUIREMENT OF THE TOMATO

To present a better conception of the growth responses of the tomato plants grown under different environmental conditions, a large amount of data was collected on the relative transpiration rates of the different cultures. The term "water requirement" of plants has been used by many investigators to express the ratio between water absorbed and dry matter produced. It is usually obtained by dividing the total units of water absorbed by the plant by the total units of dry matter produced. The data presented in table 2 on water requirement do not represent "water requirement" as generally defined, but indicate rather the relative transpiration rate of the plants in the several environments. This transpiration rate is determined by dividing the total units of water absorbed or transpired by the dry weight of the aerial vegetative part of the plants rather than by the total dry weight of all plant parts. It is well known that many factors may influence the water requirement of plants, which varies widely with different species.

As is shown by the data presented in table 2, variation in environmental conditions such as fertilizers, soil moisture, humidity, and air temperature may cause a wide variation in the efficient use of water by the plant. Low soil moisture and low air temperature have great influence in reducing the water consumption in proportion to the dry material produced; furthermore, increasing amounts of nitrogen promote greater efficiency in water utilization. In these studies plants without any added fertilizer were the most extravagant users of water.

EFFECTS OF FERTILIZERS ON ACCUMULATION OF STARCH IN TOMATO STEMS

The excessive accumulation of starch in the stems of the check plants may appear unusual and perhaps difficult to accept as truly representative, but study of the data in table 2 shows a consistent trend in the results and supports these observations. The check plants received no fertilizer, but the soil used was fairly fertile because a small amount of well-rotted manure had been added. However, a decided change in color and retarded growth indicated that these plants had used nearly all of the readily-available nitrogen when they were about half-grown. Under certain environmental conditions the accumulation of starch was most marked in the check plants; however, these plants, when grown with low soil moisture at high temperature, had less starch than the fertilized plants. We might accept the explanation that under these conditions the storage carbohydrates were largely consumed in respiration and that poor nutrition retarded their replacement. At the lowest temperature, there was marked congestion of starch in the stems even with the low soil moisture.

It is evident from these and other studies that under these experimental conditions starch congestion is more definitely related to low nitrogen supply than to differences in potash supply. No evidence of potash deficiency has been observed in any of the cultures grown

with this greenhouse soil over a period of 5 years, but numerous instances have been observed of definite exhaustion of available nitrogen. It is also apparent that other environmental factors exert considerable influence, and these will be discussed later. The influence of very marked plant-food deficiency on starch congestion has been discussed in detail by many investigators; however, little consideration has been given to the effects of approximate soil-moisture differences on plants of normal size and appearance grown in good, fertile soil.

EFFECT OF TEMPERATURE ON ACCUMULATION OF STARCH IN STEMS

The effect of temperature on the congestion of starch in stems of nitrogen-starved plants is very marked, as is shown in figure 6.

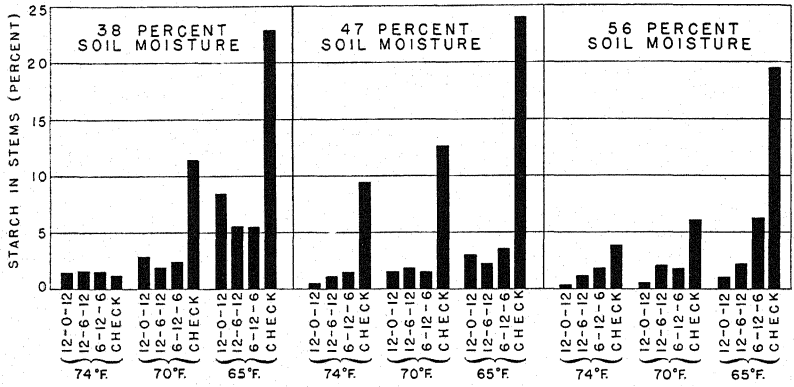


FIGURE 6.—Influence of temperature on accumulation of starch in tomato stems in various fertilized and in unfertilized cultures grown at three different levels of soil moisture.

In the 38-percent soil-moisture, no-fertilizer cultures grown in the high-temperature unit (74° F.) there was 1.27 percent of starch, an amount even lower than in any of the fertilized treatments in the same moisture and temperature series; but at 70° the starch content was 11.56 percent. At a still lower temperature (65°), the starch content was 22.9 percent, or approximately 18 times as much as at the high temperature, owing chiefly to a reduction of 9° in daily mean temperature. A difference in temperature of 9° also shows a rather significant but less marked influence on the congestion of starch in the fertilized plants in this low soil-moisture series, as further examination of the data in table 2 and figure 6 reveals.

It is interesting that 47 percent of soil moisture appeared to be optimum for starch accumulation at each temperature level in the unfertilized or check plants. However, the fertilized plants in this soil-moisture series showed relatively little fluctuation in starch content due to temperature difference. As in the case of the 47-percent soil-moisture series, the significant influence of relatively low temperature was markedly apparent in the 38- and 56-percent series (fig. 6). The temperature influence was much greater in the nitrogen-starved or check plants than in the fertilized series. In the 47-percent soil-moisture check cultures the percentage of starch was 9.4 at the high temperature, 12.7 at the medium temperature, and much higher still,

24.1, at the low temperature, or nearly three times the amount found in plants grown at the high temperature.

EFFECT OF SOIL MOISTURE ON ACCUMULATION OF STARCH IN STEMS

In figure 7 is shown the influence of soil-moisture supply on the congestion of starch in tomato stems. Differences in soil-moisture supply appear to have a very definite and significant influence on the congestion of starch in the stems, with 47 percent shown to be optimum for the check plants at each experimental temperature in these tests. No plausible explanation can be offered for this unless we consider that 47 percent of soil moisture (72 percent of the water-retaining capacity) was optimum for plant growth, and because of this condition the check plants exhausted their available nitrogen supply and accumulated more starch than the others. The wide variation in the amount of starch with respect to soil moisture in the fertilized series at the low temperature is probably due to the effect of this temperature on the

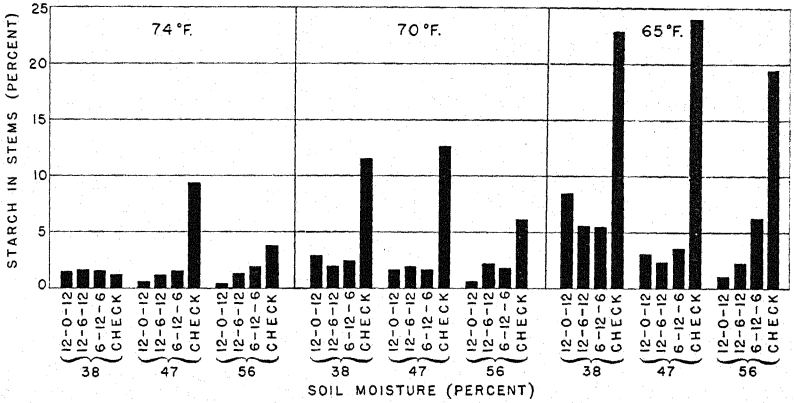


FIGURE 7.—Influence of soil moisture on accumulation of starch in tomato stems in various fertilized and in unfertilized cultures grown at three uniform temperatures.

growth activities of the plants, especially protein formation, cell division, and metabolic activity (*6*). However, since the plant response as characterized by starch congestion is so varied, a better knowledge of the interrelation and interaction of the numerous factors is necessary before an explanation can be attempted. Additional data obtained in these studies and not yet analyzed should aid in a further understanding of them.

DISCUSSION

The data presented in this paper confirm further the results obtained by Hartwell (*2*), Kraus and Kraybill (*5*), Nightingale (*6*), and many other investigators along similar lines, many of whom, however, have used different crops or methods. The experimental methods followed are similar to those followed by Nightingale (*6*) in growing tomato plants, with two major exceptions: (1) He used sand cultures and nutrient solutions, and (2) he made no attempt to determine the relation of soil-moisture supply to starch congestion or accumulation. The extreme variation in temperature conditions in the present experimental work was only 9° F., while in Nightingale's it was 41° (from 55° to 96°). The general growth habit, vigor, and plant response of

the writers' plants approached what might be called normal for field conditions (figs. 1, 2, 3, 4, and 5), while Nightingale's were stunted in comparison.

The response of the plants to the four fertilizer, three soil-moisture, and three temperature conditions, and the accumulation of starch resulting from the interaction of the various combinations of these factors, are highly significant, as shown by analysis of variance. Differences in starch congestion or accumulation at any one temperature were apparently influenced almost entirely by total nutrient deficiency and were apparently unrelated to marked differences in potash supply. In the low-temperature unit, when nitrogen metabolism and synthesis of proteins were retarded because of unfavorable temperature, there was a high accumulation of starch in the plants even though they received large amounts of nitrates. A different explanation is suggested for the results on high-nitrogen versus unfertilized check plants in the low-moisture series, when grown in the high-temperature unit. In this case the low soil moisture apparently retarded plant growth, which tended to conserve the available nutrient supply for use by the plant over a longer period; and since nutrient deficiency did not occur, accumulation of starch was about the same as in the fertilized plants. The condition of nutrient deficiency, due either to the exhaustion of the available supply or to failure on the part of the plant to assimilate because of low temperatures, shows a highly significant relation to the congestion or accumulation of starch.

The relation of low temperatures to starch congestion was clearly demonstrated by Nightingale (*6*). However, under his experimental conditions he could not observe the highly significant relation of soil-moisture supply to starch congestion. The fact that in the present experiments 47 percent of soil moisture gave the greatest starch accumulation or congestion for the unfertilized plants in each of the three different temperature units is illustrated in figure 7. This condition is thought to be due to the moisture percentage (72 percent of the water-retaining capacity) being the best of the three for plant growth; this resulted in early exhaustion of the available nitrogen, with consequent larger accumulation of starch than occurred under other moisture conditions. Further discussion of the interrelation and interaction of the various factors concerned in metabolism and synthesis and of the relation of these processes to starch congestion would best be deferred until there is a better understanding of these problems. This must await further analysis of data and perhaps further experimentation.

SUMMARY

An intensive study has been made of starch congestion or accumulation in stems of tomato plants, their growth response to various environmental influences, and the interrelation and interaction of these factors as influenced by (1) four soil-fertilizer treatments, (2) three soil-moisture conditions, and (3) three air-temperature conditions.

Starch congestion appears to be more definitely associated with nitrogen deficiency than with wide variation in potash supply, since numerous cultures high in starch have shown typical symptoms of nitrogen deficiency but no symptoms of potash deficiency.

In the unfertilized plants of the 38-percent soil-moisture series, with a decided lowering in temperature (from 74° to 65° F.) the congestion of starch increased from 1.27 to 22.9 percent.

In the unfertilized plants of the 47-percent soil-moisture series, a similar drop in temperature caused an increase from 9.4 to 24.1 percent in starch.

In the unfertilized plants of the 56-percent soil-moisture series, a corresponding drop in temperature caused an increase from 3.87 to 19.52 percent of starch.

The same lowering of temperature (9° F.) also caused an increase of starch in the fertilized plants. When the plants were grown with 38 percent soil moisture starch increased from 1.54 to 8.49 percent for the 12–0–12 fertilizer, from 1.68 to 5.61 percent for the 12–6–12 fertilizer, and from 1.59 to 5.55 percent for the 6–12–6 fertilizer.

With 47 percent of soil moisture very little congestion of starch occurred in the fertilized plants as a result of difference in temperature.

With 56 percent of soil moisture, a 9° F. lower temperature (65° vs. 74°) caused a decided increase in starch (from 1.9 to 6.32 percent) with 6–12–6 fertilizer, but less increase with 12–0–12 and 12–6–12 fertilizer.

Variations in soil moisture also appeared to have a definite influence on the accumulation of starch. With temperature constant for unfertilized plants, and increase in soil moisture from 38 to 47 percent, there was an increase in starch from 1.27 to 9.4 percent; but with further increase in soil moisture to 56 percent the starch content dropped to 3.87 percent. Forty-seven percent of soil moisture was the best of the three moisture levels for starch congestion in the check plants under all three temperature conditions. Where fertilizers were used, there did not seem to be a definite soil-moisture optimum for starch accumulation. There was some tendency for this accumulation to be greatest at the 47-percent moisture level.

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STUDIES OF SELECTED STRAINS OF CURLY TOP VIRUS[1]

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INTRODUCTION

It has been known for some time[3] that the virus causing curly top shows wide variation in virulence as evidenced by symptoms produced on European brands of sugar beet (*Beta vulgaris* L.). Similar differences in severity of symptoms were also encountered in connection with greenhouse inoculations of susceptible sugar-beet varieties obtained from breeding work of this Division. These variations in severity of symptoms were usually attributed to lack of uniformity in resistance or susceptibility in the host plant or to attenuation of the virus. It was sometimes difficult to account for the variation on either of these assumptions, and results were slightly confusing. There has also been some disagreement among investigators with respect to the curly top susceptibility of certain varieties of sugar beet, bean, and other plants.

Studies made from 1932 to 1934 on the relative resistance to curly top of sugar-beet strains arising in the breeding work of this Division indicated that there is considerable variation in the reaction within a resistant strain or "variety." This variation was attributed to lack of genetic uniformity, since it was known that the breeding material tested had been developed largely by mass selection with little inbreeding. When geneticists of the Division obtained a sugar-beet line, designated as 1167, which was much more uniform as to curly top resistance, there was better opportunity for investigating the influence of the virus itself as related to variations in curly top reaction.

EXPERIMENTS WITH SUGAR BEETS

The new, highly resistant strain 1167 has been used as the resistant material in these studies. Some of the susceptible plants used were grown from a selected strain known as 2769–24[4] and others were grown from a well-known European brand. There was little difference in the curly top reaction of the two susceptible strains and they are considered together.

Sugar-beet plants were inoculated in the cotyledon or young two-leaf stage, by placing one viruliferous beet leafhopper, *Eutettix tenellus* (Bak.), on each plant. The leafhoppers were allowed to remain on the plants for 1 week.

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[2] Acknowledgment is made to F. A. Abegg and F. V. Owen, of the Division of Sugar Plant Investigations, for constructive criticism and helpful suggestions, particularly in regard to statistical evaluation of experimental results.

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[4] 2769–24 designates a variety arising out of sugar-beet breeding work conducted by W. W. Tracy, Jr., of the Bureau of Plant Industry. Seed of this variety came as a result of direct increase of the original seed lot.

Plants were graded for severity of curly top symptoms according to a scale that ranged from 0 for no symptoms to 5 for the severest symptoms, as follows: Grade 1, either slight vein clearing or occasional papillate growths on the under side of the foliage, or both; grade 2, either slight curling of leaves with pronounced vein clearing or numerous papillae, or both; grade 3, pronounced curling and some dwarfing; grade 4, pronounced curling and increased dwarfing; grade 5, extreme curling and dwarfing. Illustrations of the type symptoms of these various grades of injury are given in plate 1 and figure 1.

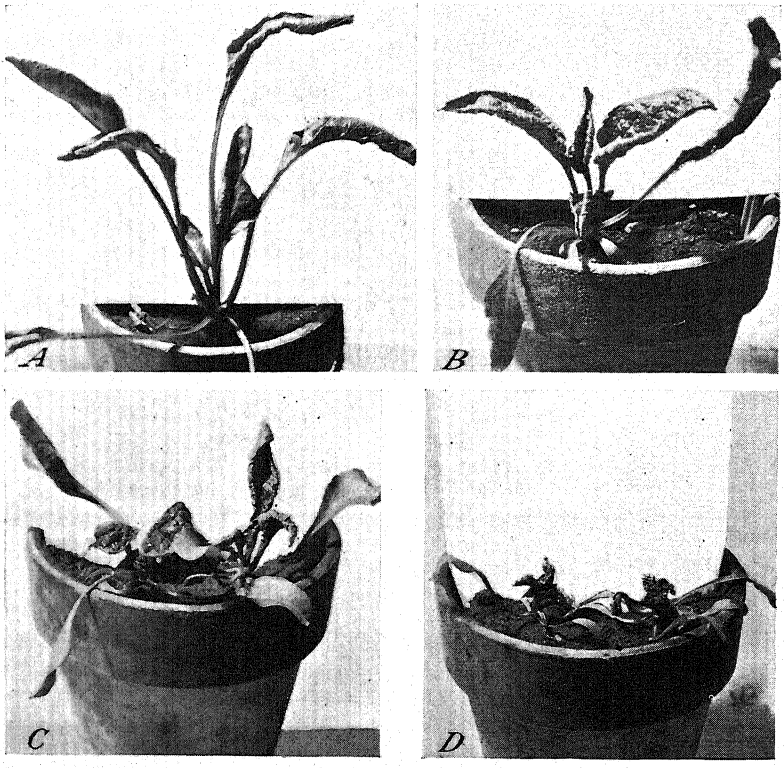
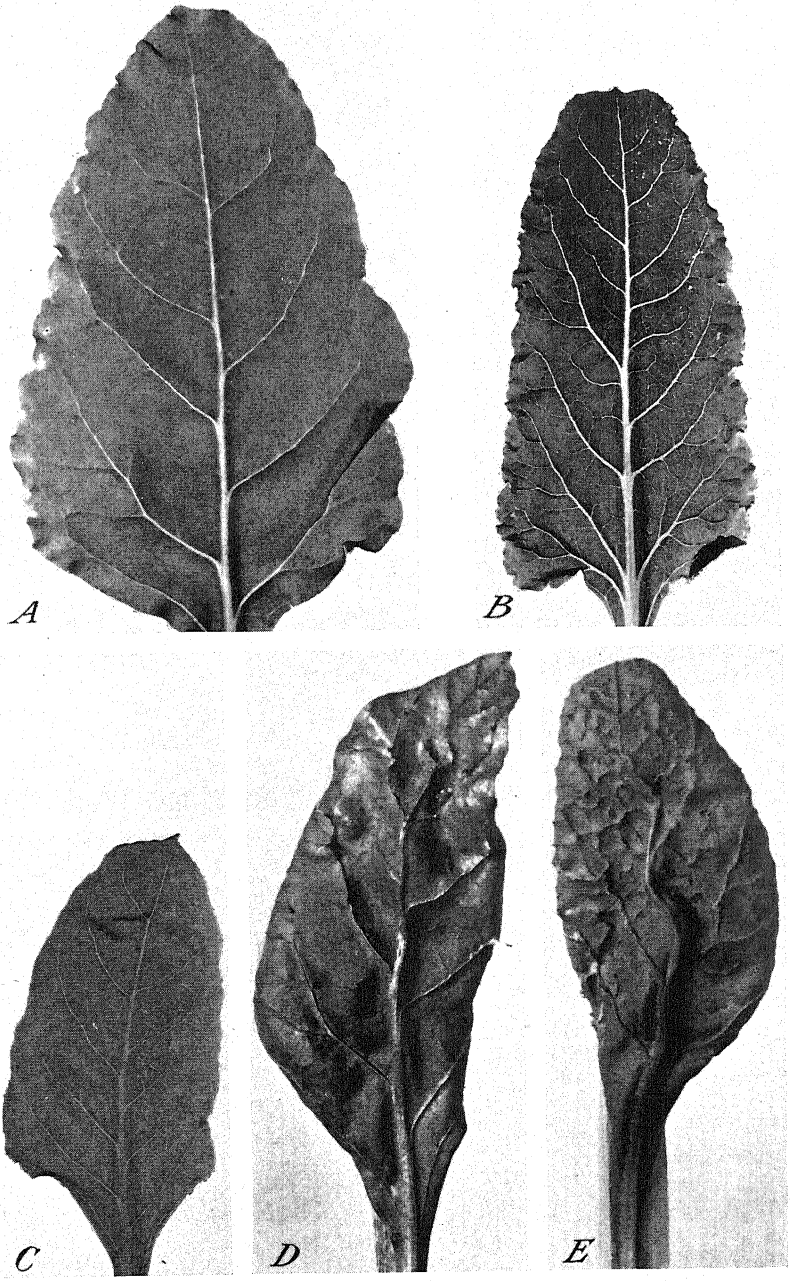


FIGURE 1.—*A*, Sugar-beet plant showing curly top symptoms that would be graded 2 in severity. There is usually pronounced vein clearing in the younger leaves, and numerous papillae, or wartlike growths, from the veins on the under side of the leaf. The leaves frequently show a tendency to roll or curl. *B*, Diseased sugar-beet plant showing pronounced leaf distortion and some dwarfing. This is classed as grade 3. *C*, Two sugar-beet plants much dwarfed and distorted by curly top. These symptoms are grade 4. *D*, Two sugar-beet plants showing extreme dwarfing and distortion. Such plants are likely to die in a short time, and the disease symptoms are recorded as grade 5.

Inoculation of resistant plants with virus from ordinary stock sources gave a wide variation in the percentage of plants infected, although the percentage was always significantly lower than for susceptible plants under similar conditions. The symptoms shown by resistant plants also differed widely, but, relatively, graded much lower than those on susceptible plants.

Some plants showing very mild symptoms and others with relatively severe symptoms were selected from the diseased resistant beets as virus sources for further inoculations, and similar selections were made from the diseased susceptible plants.



A, Normal, healthy sugar-beet leaf. *B–E*, Diseased sugar-beet leaves. *B*, Leaf of normal shape but showing vein clearing, most pronounced on right side of midvein. *C*, Slightly distorted leaf with vein clearing. Such vein clearing is quite characteristic of early symptoms in nearly all cases of curly top disease. In the very mild type of disease, there may be no other symptoms except possibly some small papillate outgrowths from the veins on the under side of the leaf. Plants showing symptoms to this extent, or less, are classed as grade 1. *D* and *E*, Diseased sugar-beet leaves showing large and much elongated papillate growths on under side of leaf; *D* might be graded 1 but *E* would grade 2.

Successive inoculations of the resistant and the susceptible test plants with virus from 94 such selected virus sources soon established the fact that the virus isolants could be classed in three major groups, as evidenced by distinctive reactions on the host plants. One or more plants from each group were selected as virus sources for further inoculations. For convenience in discussion, the virus isolants thus obtained will be referred to as virus strains, with the understanding that they may be further resolvable by appropriate technique.

Susceptible beets were readily infected by all three of the above virus strains, one inducing severe symptoms and the other two inducing mild symptoms. Resistant beets were quite easily infected by the strain that induced severe symptoms in the susceptible beets and by one of the two strains that induced mild symptoms in the susceptible beets, but they were quite resistant to infection by the other strain. Selections and inoculations have now been continued over a period of 2 years, and it has been found possible to differentiate clearly the symptoms induced by a fourth isolant.

In the earlier experiments, only three virus strains were included in each test and there was no replication as to virus source. In later work, the test of a virus strain was replicated by use of two or more different plants from a previous set of inoculations as independent virus sources. Each such replication is listed separately. Seven tests included all four virus strains at one inoculation, one test included only two, and the others included three virus strains variously chosen.

It seems that more reliable information is obtainable by consideration of all data for a strain as a whole, rather than by consideration of the separate comparative tests. The data as given in table 1 group plants inoculated at one time with a given strain. The uniformity of behavior is quite evident from the columns showing plants infected and grade of severity of symptoms. Considering the possibilities of variation in the plants, the environment, the virus, and the vector, the number of plants or experiments showing significant divergence from the general average is comparatively slight.

In the resistant beets, virus strain 1 shows 74 plants graded 3 and none graded 4 or 5; strain 2 shows only 5 plants graded 3 and none graded 4 or 5; and strains 3 and 4 show practically none above grade 1. In the susceptible beets, virus strains 1 and 3 show practically no plants in grades 1 or 2; strain 3 has relatively few in grade 3; and strains 2 and 4 have very few plants in grade 4 and none in grade 5.

The χ^2 test was used to determine statistically the relative significance of the differences between any one of these virus strains and each of the others. The computed χ^2 values are given in table 2.

The data from resistant beets, including all inoculated plants (grades 0 to 5, inclusive), show very highly significant differences among the virus strains. When severity of symptoms on infected plants only (grades 1 to 5, inclusive) is considered, the resistant beets show no significant difference between strains 3 and 4, but the differences between these two strains are brought out as highly significant by the susceptible beets in both the infected group and the group showing plants not infected. On the other hand, there are no significant differences between strains 2 and 4 in either group of susceptible plants, but highly significant differences between them do appear in the resistant groups.

TABLE 1.—*Frequency of occurrence of grades of severity of curly top symptoms in susceptible and in resistant sugar-beet plants when infected by virus strains 1, 2, 3, or 4, from July 1934 to February 1936, inclusive*

| Date | Number of resistant and susceptible plants showing indicated grade of severity of symptoms after inoculation with— | | |

| | | |

TABLE 2.—*χ^2 test as a means of estimating the significance of differences in curly top grade frequencies in sugar beets following inoculation of the test strain with various virus strains*

Range of curly top grades compared	χ^2 values from comparisons of virus strains—											
	1 and 2		1 and 3		1 and 4		2 and 3		2 and 4		3 and 4	
	Observed	Required [1]	Observed	Required [1]	Observed	Required [1]	Observed	Required [1]	Observed	Required [1]	Observed	Required [1]
RESISTANT BEETS												
1 to 5, inclusive	289.9	9.2	184.7	6.6	250.6	6.6	13.8	6.6	21.6	6.6	0.0	6.6
0 to 5, inclusive [2]	292.7	11.3	56.9	6.6	354.6	9.2	424.0	6.6	167.7	9.2	46.1	6.6
SUSCEPTIBLE BEETS												
1 to 5, inclusive	784.9	6.6	33.7	6.6	684.1	6.6	548.8	6.6	6.8	9.2	415.6	6.6
0 to 5, inclusive [2]	787.5	9.2	34.4	13.3	636.3	9.2	548.1	9.2	10.7	11.3	437.0	9.2

[1] For $P=0.01$.
[2] By including grade 0, the percentage of plants infected becomes a factor.

Table 3 presents a summary of the data from table 1. In this table the percentage of plants infected (ability of the virus to infect) is considered under the heading "Total plants," and the most valuable differential data for that group are in the percentage column of the resistant plants. The χ^2 test shows no significant difference between virus strains 1 and 2 in ability to infect resistant beets, but an extremely high significance between either 1 or 2 and 3 or 4. The difference between strains 3 and 4 is also highly significant. The virus strains show no significant differences in ability to infect the susceptible plants.

TABLE 3.—*Summary of percentages of infection and of curly top frequency grades, and average grade of disease severity in susceptible and in resistant sugar-beet plants when infected by different virus strains*

Virus strain No.	Tests	Total plants						Infected plants showing grade of severity of symptoms [1]										Average grade of severity for diseased plants	
		Resistant				Susceptible		Resistant					Susceptible						
		Inoculated	Infected			Inoculated	Infected											Resistant	Susceptible
		No.	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*		
1	51	1,067	683	64		893	708	79	18	71	11	0	0	0	2	16	39	43	1.9	4.3
2	29	592	360	61		527	423	80	72	26	1	0	0	7	75	17	1	0	1.3	2.1
3	33	773	67	9		505	409	81	94	3	3	0	0	0	1	8	31	60	1.1	4.5
4	21	454	103	23		365	275	75	94	6	0	0	0	2	76	21	1	0	1.1	2.2

[1] Percentages are based in each case on the total number of diseased plants among those that had been inoculated with the specified virus strain.

In any comparative study of differential reaction to the curly top virus, severity of symptoms is an entirely different criterion from percentage of infection, and in table 3 the diseased plants are considered

as a distinct group under the heading "Infected plants." The percentages given here are based on the total number of diseased plants among those inoculated with the specified virus strains. Considering the resistant beets, virus strain 1 was the only strain inducing grade 3 symptoms in an appreciable percentage of plants, and its ability to infect was the highest of any; strain 3 showed very low ability to infect (9 percent as compared with 64 percent for strain 1), and 94 percent of all infected plants graded 1 in severity. Virus strain 1 also induced severe symptoms on susceptible beets, but strain 3 gave a significantly higher percentage in the grade 5 class and a higher average severity of symptoms. From all data on resistant beets, virus strain 1 would be classed as far more virulent than strain 3; while the data on susceptible beets would indicate strain 3 as the more virulent.

All four virus strains produced high percentages of infection in susceptible beet varieties. Strain 1 induced severe symptoms in the susceptible variety and a high percentage of infection with mild to intermediate severity in the resistant variety. Strain 2 induced mild symptoms in the susceptible variety and a relatively high percentage of infection but very mild symptoms in the resistant variety. Strain 3 produced extremely severe symptoms in the susceptible plants but rarely infected a plant of the resistant strain, and any resistant beets infected developed very mild symptoms. Strain 4 gave mild symptoms in the susceptible variety and a small percentage of infection with very mild symptoms in the resistant variety.

The four virus strains may be roughly grouped according to the plan given in table 4, in which plus signs (+) indicate high percentages of infection or relatively severe symptoms and minus signs (−) indicate low percentages of infection or relatively mild symptoms. This grouping is based upon data from a large number of comparative inoculation experiments. The smallest number of plants involved was 365 in the case of susceptible plants tested with virus strain 4, while the largest number was 1,067 of the resistant plants tested with virus strain 1.

TABLE 4.—*Classification of virus strains according to ability to infect and severity of symptoms in sugar beets* [1]

Virus strain No.	Relative percentage of infection—		Relative severity of symptoms in—	
	Susceptible beets	Resistant beets	Susceptible beets	Resistant beets
1	+	+	+	+
2	+	+	−	−
3	+	−	+	−
4	+	−	−	−

[1] Plus signs (+) indicate high percentages of infection or relatively severe symptoms; minus signs (−) indicate low percentages of infection or relatively mild symptoms.

The percentages of plants showing different grades of severity of symptoms are presented graphically in figure 2.

Virus strains 1, 2, and 4 were readily maintained as "cultures" in either the susceptible or the resistant beets, but some difficulty was

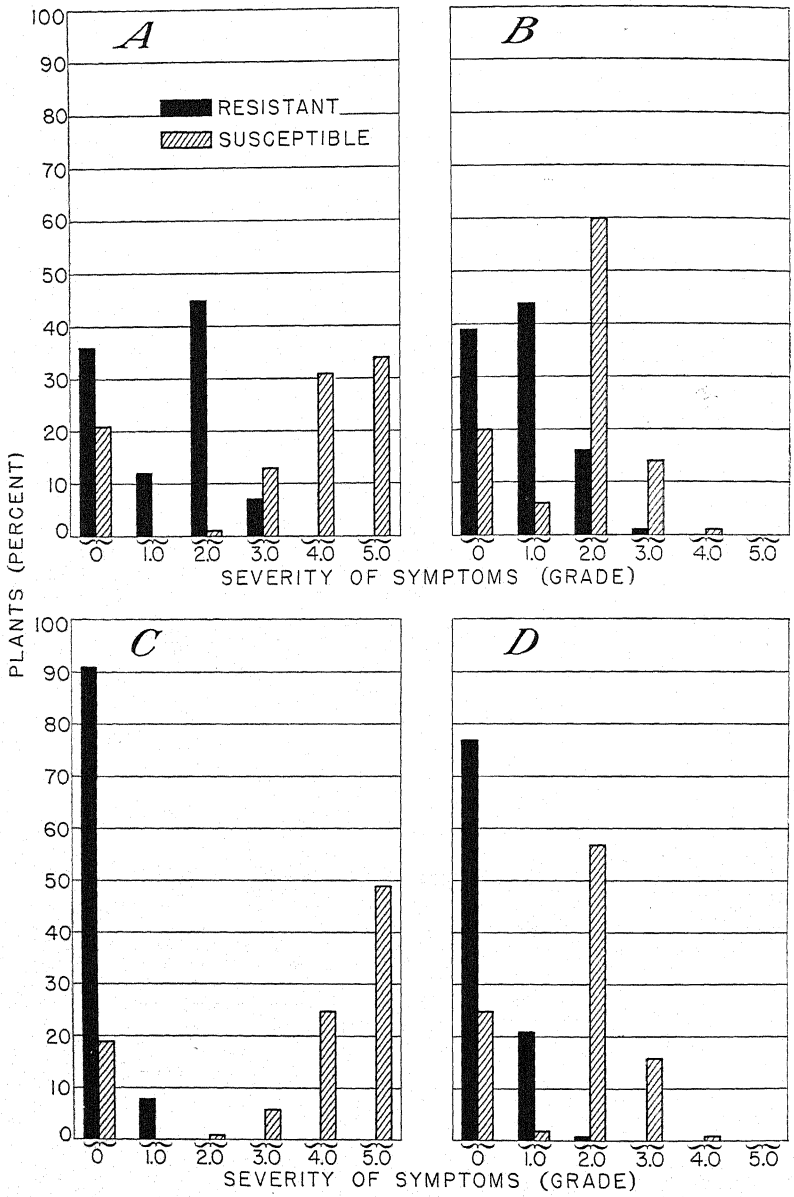


FIGURE 2.—Percentages of sugar-beet plants showing different grades of severity of symptoms after inoculation with different curly top virus strains. (0=no infection.) *A*, Virus strain 1; *B*, virus strain 2; *C*, virus strain 3; *D*, virus strain 4.

experienced in the case of strain 3 because the young susceptible plants were often so severely injured that they died and very few of the resistant plants became infected. It was also found that virus strain 3, when obtained from resistant beets, frequently had undergone a change in virulence which appeared to be permanent. Further studies regarding virus changes, with possible relation to virus strains, are in progress.

REACTIONS OF OTHER HOSTS TO THE VIRUS STRAINS

These four strains of curly top virus have been tested on tomato *Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), bean (*Phaseolus vulgaris* L.), plantain (*Plantago erecta* Morris), and peppergrass (*Lepidium nitidum* Nutt.)

It may be noted that the numerous hairs on tomato, tobacco, and bean seem to entangle and irritate the leafhoppers, resulting in their early death. Scraping the leaves gently with a safety-razor blade or rubbing them with cloth to remove many of the hairs resulted in a greatly decreased mortality. Unless otherwise noted, four leafhoppers per plant were used on tomato, bean, and tobacco, and one leafhopper per plant on plantain and peppergrass. The leafhoppers were allowed to remain on the plants for a week.

The Stone variety of tomato was used, and the plants were inoculated when they were 3 to 4 inches tall. The results are given in table 5. Virus strains 1 and 3 gave fairly high percentages of infection, while strains 2 and 4 gave no infection.

Inoculations into Turkish tobacco furnished data that are likewise presented in table 5. The plants were inoculated when they were 2 to 6 inches tall. Four leafhoppers per plant were used for the January inoculation, and eight per plant for the March and May inoculations. The greater effectiveness of the larger number of insects is clearly apparent. Percentages of infected plants in this host closely parallel those for tomato. Neither virus strain 2 nor strain 4 produced any symptoms on tobacco, and numerous attempts to recover the virus from such inoculated plants gave negative results.

Three varieties of bean were used, and inoculations were made before the second leaf had opened out. The Red Mexican variety was selected for extremely high resistance, and there was no evidence of infection on any of the inoculated plants. Attempts to recover the virus from these plants gave negative results.

Two selected strains of Great Northern, namely, the Ellsworth strain and the University of Idaho No. 81 strain, were selected for intermediate resistance. There was no evidence of any significant difference in the reactions of these two strains of bean, and the results from both are included in table 5. Virus strain 4 gave a small amount of infection in these beans, while strain 2 gave no infection. The Great Northern bean variety, the plantain, and the peppergrass are the only hosts so far tested, except beet, that have served to differentiate virus strains 2 and 4.

The results of inoculation of Bountiful beans are given in table 5. There was a high percentage of infection with each of the four strains of virus, but the symptoms induced by strains 1 and 3 were markedly more severe than those induced by strains 2 and 4. Infection by strains 1 and 3 resulted in early loss of leaves, complete cessation of growth, and relatively early death.

The plantain (*Plantago erecta*) plays a very important role in the life of the sugar-beet leafhopper under California conditions, and its reaction to the different virus strains is of particular interest. The plants were inoculated in the two- to four-leaf stage. Difficulty has been experienced in growing this plantain under greenhouse conditions and in being certain as to symptoms. It seems necessary to rely upon curling and twisting of the leaves as symptoms in both the plantain

and the peppergrass. The severe cases are easily noted, unless the plant dies without making appreciable growth after inoculation, but slight curling or twisting may be confused with some other type of injury or with normal growth changes. Table 5 gives the data thus far secured.

TABLE 5.—*Effect on various hosts of inoculations with different strains of virus of curly top of sugar beet*

Variety and date	Strain 1			Strain 2			Strain 3			Strain 4		
	Plants inoculated	Plants infected		Plants inoculated	Plants infected		Plants inoculated	Plants infected		Plants inoculated	Plants infected	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
TOMATO												
Stone:												
June 20, 1935	60	26	43	60	0	0	40	19	48	20	0	0
July 26, 1935	112	82	73	80	0	0	40	19	48	20	0	0
May 28, 1936	36	11	31	16	0	0	52	18	35	17	0	0
Total	208	119	57	156	0	0	132	56	42	57	0	0
TURKISH TOBACCO												
Jan. 17, 1936	19	7	37	19	0	0	57	18	32	19	0	0
Mar. 3, 1936	71	50	70	20	0	0	53	31	58	12	0	0
May 28, 1936	40	33	83	8	0	0	60	44	73	20	0	0
Total	130	90	69	47	0	0	170	93	55	51	0	0
BEAN												
Great Northern:												
July 27, 1935	65	28	43	34	0	0	33	4	12	10	1	10
Sept. 10, 1935	60	18	30	24	0	0	57	27	47	2	1	50
July 11, 1936	76	16	21	0	0	0	341	184	54	36	3	8
Total	201	62	31	58	0	0	432	215	50	48	5	10
Bountiful:												
July 27, 1935	58	[1] 43	74	32	21	66	32	[1] 28	88	8	8	100
PLANTAIN												
Nov. 27, 1935	11	6	55	1	0	0	11	8	73	10	1	10
Feb. 7, 1936	54	32	59	29	0	0	43	35	81	8	0	0
Mar. 23, 1936	189	135	71	76	0	0	199	124	62	56	2	4
May 1, 1936	65	54	83	32	0	0	99	87	87	33	0	0
Total	319	227	71	138	0	0	352	254	72	107	3	3
PEPPERGRASS												
Nov. 27, 1935	112	50	45	41	0	0	74	65	88	38	1	3
Feb. 7, 1936	87	54	62	46	0	0	69	64	93	20	1	5
May 1, 1936	55	41	75	27	0	0	81	68	84	26	2	8
Total	254	145	57	114	0	0	224	197	88	84	4	5

[1] About 70 percent of the Bountiful bean plants infected with virus strains 1 or 3 lost their terminal buds and ceased all growth in less than 3 weeks after inoculation.

Some infection was obtained with virus strain 4 but none with strain 2, while relatively high infection percentages were secured with strains 1 and 3.

To study these strains of virus in *Plantago* under more nearly natural conditions, inoculations were made upon plants grown in cages in the field. The plantings were made in January, which was rather late for satisfactory results. Only three virus strains (1, 3, and 4) were used. The plants were grown under cloth-covered cages 18 inches square. When the plants were 6 weeks old, those in each cage were inoculated with one of the three virus strains used. These plants were tested in May, and each strain was recovered from the group that had been inoculated with it.

The native peppergrass is also an important California host of the leafhopper. Inoculation results (table 5) were very similar to those from plantain. The percentage of plants infected with strain 4 was very low, and strain 3 gave a much higher rate of infection than strain 1. Because of the occasional symptoms produced in plants inoculated with virus strain 4, it seemed desirable to test a number of the plants inoculated with strains 2 and 4. Nineteen plants inoculated with strain 4 and 28 plants inoculated with strain 2 were tested by placing nonviruliferous leafhoppers on them and then transferring these leafhoppers to susceptible beets. In no case was there evidence of beet infection unless the peppergrass had been graded as diseased.

DISCUSSION

It seems clearly demonstrated that curly top virus is a complex of strains that can be separated into recognizable entities by differential host-plant responses. In this study four such strains have already been differentiated, and, considering the limited number of hosts used and virus sources tested, it appears certain that many other curly top virus strains will eventually be recognized. The existence of strains within the curly top virus may have a significant relation to breeding which seeks to develop further, in sugar beet or in other plants, varieties resistant to curly top. It is conceivable that a virus strain which had not previously been a factor might suddenly find favorable conditions for widespread development with more or less disastrous results. There are some sugar-beet varieties and many varieties of other economically important crop plants that have not been tested with the four virus strains described in this paper. Their response to inoculation with any of these four virus strains cannot be predicted.

In field studies or in commercial sugar-beet fields, the prevalence of one or another strain of virus might result in quite different symptoms and injury. It is highly important to learn the factors that influence the predominance of any strain of virus in certain areas or during certain seasons. Such data are being sought by field and greenhouse studies of the virus strains in relation to native and overwintering curly top host plants.

These studies furnish at least a partial explanation of the inconsistent or divergent results occasionally secured in curly top studies. An investigator testing virus strain 3 on resistant sugar beet 1167 would certainly conclude that this variety is practically immune to curly top, but if the inoculum happened to be strain 1 this resistant variety would be rated as quite susceptible to infection and subject to appreciable injury. Similar discrepancies might easily occur as a result of work with any of the strains described.

The more virulent virus strains, 1 and 3, induce extremely severe symptoms on the most important overwintering hosts, *Lepidium*, *Plantago*, and filaree. The young plants are often killed or reduced to a very small, inconspicuous tuft of growth. This fact suggests that these highly virulent strains tend to be self-limiting under certain conditions and it may help to explain the greater prevalence of the less virulent strains during some seasons or in some areas.

CONCLUSIONS AND SUMMARY

Four curly top virus strains have been recognized by the differential reactions of sugar beets, percentages of plants infected and severity of symptoms being used as the bases of comparison. Beets of the resistant line 1167 showed distinctive reactions to each of the four virus strains, and the susceptible beets gave reactions that differentiated strain 1 from strain 2, 1 from 3, 1 from 4, 2 from 3, and 3 from 4. Virus strains 2 and 4 could not be separated by the responses of the susceptible test beets.

In susceptible beets, virus strains 1 and 3 induced severe symptoms, whereas strains 2 and 4 produced only mild symptoms. The percentages of susceptible plants infected were approximately the same for all four virus strains.

In beets of the resistant line 1167, virus strain 1 induced obvious (not severe) symptoms, but strains 2, 3, and 4 produced only inconspicuous symptoms. The percentages of resistant beets infected by virus strains 1 and 2 were high, whereas for strains 3 and 4 they were very low.

The more virulent strains, 1 and 3, were easily distinguished from the less virulent strains, 2 and 4, by inoculation into tobacco or tomato; but these hosts did not differentiate strain 1 from 3 or 2 from 4.

The bean varieties, as well as *Plantago erecta* and *Lepidium nitidum*, did not give responses that were satisfactory for distinguishing strain 1 from strain 3, although *Lepidium* gave a significant difference between strains 1 and 3 in the percentages of plants infected.

These experiments show that the curly top virus exists as strains which vary in ability to infect and injure different hosts.

MOSAIC OF LIMA BEANS (PHASEOLUS LUNATUS MACROCARPUS) [1]

By L. L. HARTER

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INTRODUCTION

In 1936 Harter (*4*)[2] published an abstract calling attention to a mosaic of lima beans (*Phaseolus lunatus macrocarpus* Benth.)[3] that appeared in Maryland in the summer of 1935. It was stated therein that, while the symptoms of the disease somewhat resembled those of the common bean mosaic, it could not be transmitted to beans (*P. vulgaris* L.). For this and other reasons, it was believed to be different from bean mosaic and from the mosaics of other legumes. Certain symptoms obtained from inoculating the lima bean mosaic virus into tobacco (*Nicotiana tabacum* L.), cucumber (*Cucumis sativus* L.), and broadbean (*Vicia faba* L.) suggested its possible identity with or close relationship to the cucumber mosaic virus.

Specimens of mosaic-infected lima beans were collected in the breeding grounds of the Division of Fruit and Vegetable Crops and Diseases located near Beltsville, Md., from a mixed population of hybrid progenies of lima beans. Five to ten percent of the plants were diseased and were scattered more or less indiscriminately over the field. The first symptoms appeared in a mild form in July 1935. The number of diseased plants and the severity of the symptoms increased as the season advanced. A detailed study of lima bean mosaic was begun in September of the same year, when a number of infected plants were removed from the field to the greenhouse to provide a source of mosaic material for further study. These plants flourished under greenhouse conditions and in a few weeks produced an abundance of foliage showing the characteristic field symptoms of mosaic.

The same symptoms were observed again in 1936 in the lima bean breeding trials, although a number of the more susceptible progenies had not been planted.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Lima bean mosaic has been reported from widely separated sections of the country. McClintock (*6*) reported its occurrence on several varieties of lima beans of the sieva type at Norfolk, Va., in 1916. It was not found on any of the large-seeded Fordhook types. He concluded that, since lima beans had not been grown on the land previous to 1916, the infection was carried by the seed. Adams (*1*) briefly stated that a lima bean mosaic was generally distributed in Delaware in 1923 and that it was of increasing importance in that State. Osner

[1] Received for publication September 2, 1937; issued June, 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 905.

[3] In ordinary botanical usage, *Phaseolus lunatus* is the civet or sieva bean and *P. lunatus macrocarpus* the lima bean.

(*8*) listed it among the diseases of economic importance in Indiana in 1917, and Harrison [4] reported its occurrence on several varieties in New York in 1936. The writer observed the mosaic on several varieties in Michigan in 1926, and McCubbin has reported (though not published) its occurrence on the Challenger lima in Cuba. Cook (*2*) listed the lima bean among the crops of Puerto Rico attacked by a virus which he suspected might be the same as the bean mosaic. It was not seed-borne, and *Aphis rumicis* L. was thought to be a possible vector. These reports of the wide geographical distribution of the disease indicate that its presence in any part of the country should occasion no surprise.

No estimates are available as to the losses caused by the lima bean mosaic. Reports of its increasing economic importance and general observations indicate that it is on the increase and may eventually become a serious menace to the crop if susceptible varieties are more widely grown.

MATERIALS AND METHODS

SOURCE OF VIRUSES

The lima bean virus used in these experiments was obtained originally from field plantings near Beltsville, Md., or from plants grown in the greenhouse, where conditions could be controlled and the plants protected against contamination by insects carrying the virus from other susceptible hosts. The virus is very potent in the leaves, and careful comparison of infected field- and greenhouse-grown plants showed the symptoms to be identical.

Because of the close similarity of the lima bean mosaic to the mosaic of cucumbers (*3*), the two causal viruses have been carefully compared.[5] To do this cross inoculations were made and a number of hosts susceptible to the cucumber mosaic were inoculated with the virus from lima beans.

METHODS

The method of inoculation employed was the one generally used in the mechanical transmission of viruses and was briefly as follows: A supply of viruliferous material was maintained in the greenhouse by inoculation of lima beans and Turkish tobacco (*Nicotiana tabacum*). The inoculum was taken from the leaves of one or the other of these hosts. The leaves were thoroughly macerated and the juice was squeezed out through cheesecloth. To the liquid was added about 2 volumes of water to 1 of plant juice. The ratio of water to plant juice is not important, provided the dilution is not too great. Preparatory to inoculation, the leaves were dusted with a small quantity of finely pulverized carborundum powder. The inoculations were made by rubbing the upper side of the primary leaves with a small quantity of absorbent cotton wrapped in cheesecloth that had been dipped in the inoculum. The best results are obtained by rubbing only sufficiently hard to produce slight abrasions of the leaf. A few minutes after the leaves were rubbed, most of the inoculum was removed by spraying the leaves with a small amount of water. These methods have been

[4] HARRISON, A. L. VARIETAL SUSCEPTIBILITY OF LIMA BEANS TO MOSAIC. U. S. Bur. Plant Indus. Plant Disease Reptr. 20: 291. 1936. [Mimeographed.]

[5] The cucumber virus and the celery virus, which it closely resembles and which was used to a limited extent in a comparative study, were supplied by S. P. Doolittle, of the Division of Fruit and Vegetable Crops and Diseases.

followed more or less closely throughout these investigations and have given highly satisfactory results.

HOST RANGE

Hundreds of lima beans, including most of the different varieties, and a considerable number of other plants have been inoculated with the lima bean mosaic virus. A sufficient number of different genera were employed to show that the lima bean mosaic virus will infect hosts widely separated in relationship. Because of the apparently close relationship of the lima bean mosaic to the cucumber mosaic, some of the plants known to be susceptible to the latter were used for comparison. The results of the host-range studies appear in the following tabulation:

Plants inoculated:	Reaction [1]	Plants inoculated—Cont'd.	Reaction [1]
Cucumis sativus L.	+	*Datura stramonium* L.	−
Nicotiana tabacum L. (Turkish)	+	*Polygonum aviculare* L.	−
Phaseolus vulgaris L. (Refuge)	−	*Petunia hybrida* Vilm.	+
Zinnia elegans Jacq. (Double Fantasy)	+	*Vicia faba* L. (Broadbean)	+
Lycopersicon esculentum Mill. (Globe)	+	*Vigna sinensis* Torner (Black and Blackeye)	+
Pisum sativum L. (Harrisons Glory)	−	*Trifolium hybridum* L.	−
Lathyrus odoratus L.	−	*Vicia sativa* L.	−
Phytolacca americana L.	+	*Vicia villosa* Roth	−
Solanum melongena L. (Superior High Bush)	+	*Lens esculenta* Moench	−
Capsicum annuum L. (World Beater)	+	*Phaseolus calcaratus* Roxb.	−
		Lupinus albus L.	−
		Soja max (L.) Piper	−
		Zea mays L.	−
		Zea mays everta Bailey	+

[1] Minus signs (−) indicate resistance; plus signs (+), susceptibility.

SYMPTOMATOLOGY

LIMA BEAN

The symptoms of the lima bean mosaic can be described best by tracing the changes that take place in the plant, especially in the leaves, from the first visible symptoms progressively through the different stages of growth to the mature leaves.

Similar to the mosaics of the many other hosts, the earliest symptoms on lima bean are characterized by a slight clearing of the veins of the juvenile leaves, which is soon followed by some very striking leaf symptoms. The lamina of the leaflet may bend downward to an angle of 45° or more, accompanied at the same time by a recurving downward of the midrib. Frequently, the plant dies in this stage. If, however, the attack is not severe enough to kill the plant, the leaflets and the midribs in the processes of growth gradually resume their normal position. At this time or when the leaflets and midribs are still curved downward, the vine grows from 1 to 2 inches long. The apical end of the vine often curves downward instead of assuming the erect position of a normal plant. The second pair of unfolding leaflets hang limply from the pulvinus.

Inoculations were made by rubbing inoculum into the primary leaves at about the time they were fully grown or 1 or 2 days earlier; i. e., just before any vine growth had started. Under the most favorable conditions, symptoms appear in 5 to 6 days. If the plant survives the first onslaught of the disease, vine growth starts and a full-sized

plant may eventually result although the rate of growth is considerably retarded. The drooping leaves slowly return to their normal position, and a slight yellow flecking may be observed at about this time (pl. 1, *A*). Concomitantly with the further growth of the plant the flecks on the leaves increase in number and size (pl. 1, *B* and *C*). Sometimes, instead of flecks, larger light-colored areas of various sizes occur, often, though not always, near the margin (pl. 1, *D* and *E*). By the time the leaves reach maturity the flecks combine to form alternate light and dark spots characteristic of mosaic in many other plants. Irregularities in the growth of different portions of the leaf, or other causes, result in the production of islands of raised or depressed spots (pl. 1, *F* and *G*), of various sizes, composed of dark-green tissue. The entire series of changes and symptoms is completed by the time the host has developed the third or fourth set of trifoliate leaves.

Occasionally primary lesions occur on the inoculated primary leaves. They are characterized by a clearing of the veins and veinlets. This is accompanied by the development of spots, in the form of a distinct yellow mottling, that are distributed more or less uniformly over the entire leaf. The spots are indeterminate in outline and bear no direct relation to the veins. The evidence of primary leaf infection occurs in about 5 days after inoculation.

The lima bean virus has not been found to produce any symptoms on the pods.

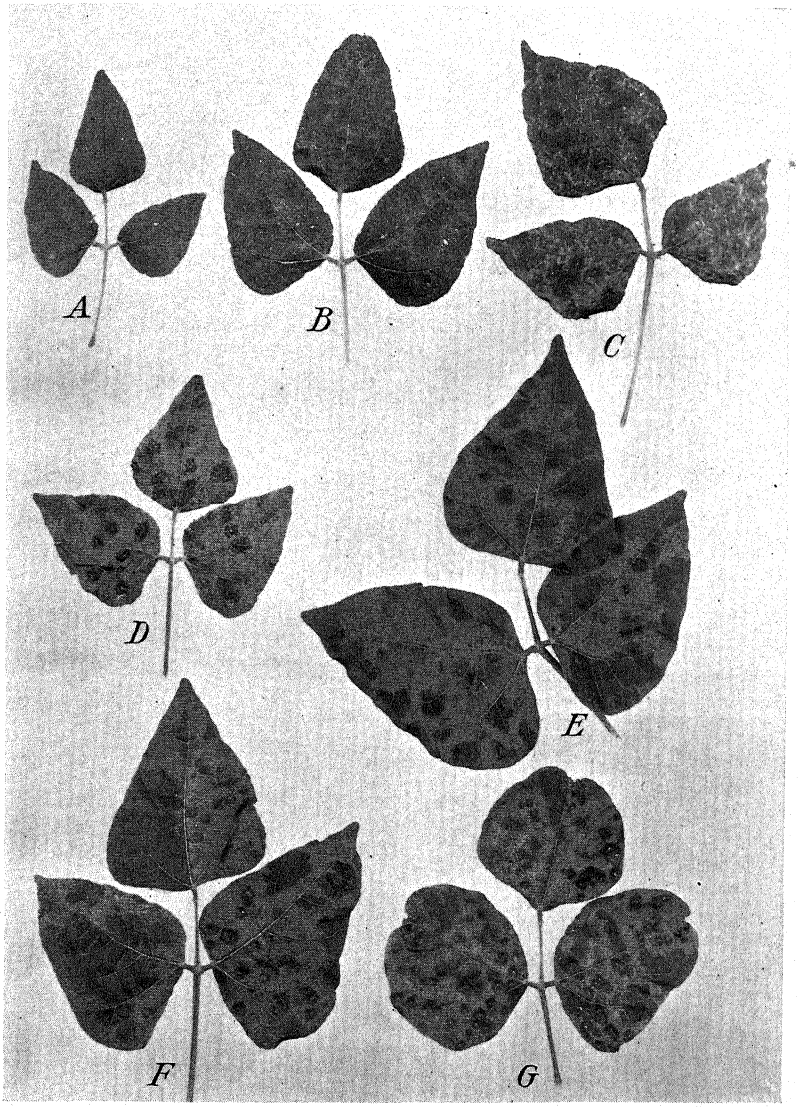
COWPEA

Only local lesions are produced by the lima bean virus on the Black and on the Blackeye cowpea (*Vigna sinensis*). These lesions are visible in 48 hours, and under favorable conditions (80°–100° C.) in 36 to 40 hours. They appear as minute specks (pl. 2, *I*) that gradually enlarge and become somewhat irregular in outline. When fully developed they range from 0.5 to 3.5 mm in diameter. These specks are purplish in color and sometimes are surrounded by a narrow band of light-green tissue. The centers of the spots are sometimes gray in color. Local lesions may be produced on the trifoliate leaves, though they develop there somewhat more slowly than on the primary leaves.

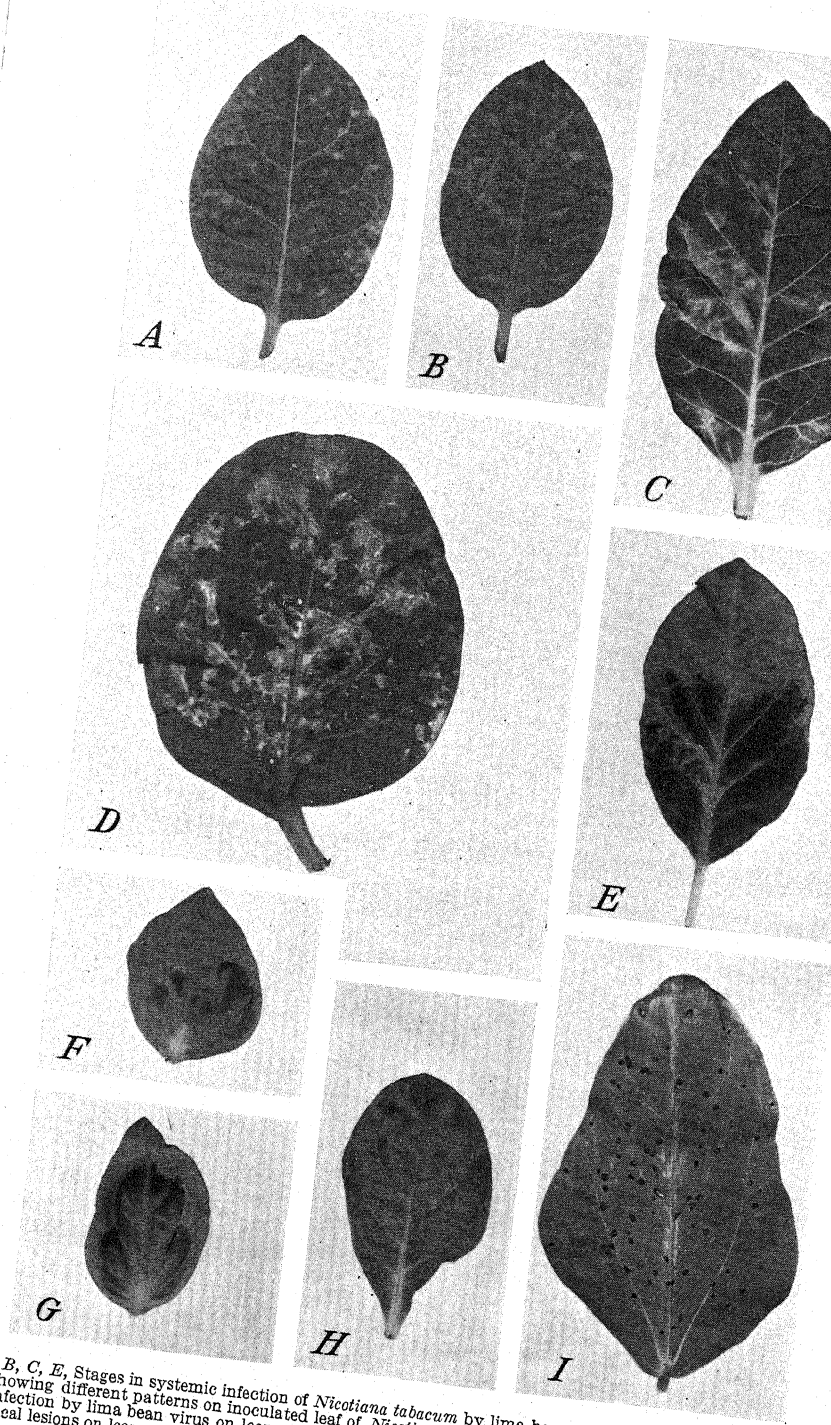
The local lesions on the Black and on the Blackeye cowpea, which for the most part are very similar, differ in one or two details. The Black cowpea lesions continue to increase in size for several days or until they attain a diameter of about 3.5 mm, whereas the lesions on the Blackeye cowpea remain small, usually not exceeding 1 or 2 mm in diameter. The difference in the size of the lesions is very consistent and apparently is strictly a varietal characteristic.

TOBACCO

The lima bean virus on tobacco (*Nicotiana tabacum*) causes symptoms that are characterized by retarded growth and a clearing of the veins, accompanied by a slight yellowing of the young leaves 5 to 7 days after inoculation. These early symptoms are frequently followed by the development of small yellowish spots (pl. 2, *A*) arranged more or less uniformly over the entire leaf. The spots gradually enlarge, some coalescing with others to form areas of various sizes and shapes (pl. 2, *B*). No further changes occur on these leaves but those subsequently developed may acquire two distinct patterns, one in which there is a yellowing in large localized areas, circumscribed to some extent by the large lateral veins (pl. 2, *C*), and the other in which



A–G, Stages in the development of lima bean mosaic on lima beans.



A, B, C, E, Stages in systemic infection of *Nicotiana tabacum* by lima bean virus; *D,* primary infection, showing different patterns on inoculated leaf of *Nicotiana tabacum*; *F, G, H,* stages and symptoms of infection by lima bean virus on leaves of *Petunia hybrida*; *H,* first visible symptoms; *I,* three-day-old local lesions on leaves of *Vigna sinensis* (Black variety).

large portions of the tip (pl. 2, *E*) or other parts of the leaf may be involved irrespective of the veins. These symptoms are not very different, if at all, from those caused by the cucumber virus.

Primary lesions generally result on the inoculated leaf of tobacco. These lesions are distributed more or less evenly over the leaf and vary greatly in form and size. Rings or circles of light-green tissue sometimes form a portion of the mosaic pattern. The circular and other spots and patterns are outlined by finely etched lines that differ markedly in general appearance from the mosaic symptoms on other leaves of the plant. The assortment of patterns is illustrated by plate 2, *D*.

PETUNIA

The leaves of young plants of petunia (*Petunia hybrida*) inoculated with the lima bean virus are slow to develop characteristic symptoms. Some stunting is evident in about 10 days, and soon thereafter the leaves show an unhealthy condition. Light-yellowish spots (pl. 2, *H*), indistinct at first, appear distributed more or less uniformly over the entire leaf.

When the plants become older, the characteristic symptoms are very much changed. The mild mottling of young plants is replaced on the leaves of older ones by islands of badly malformed tissue, in which apparently unequal growth of cells results in the production of dark-green blisterlike spots of various sizes. These malformed areas do not usually extend to the margin (pl. 2, *F* and *G*) of the leaf, although a considerable portion of the leaf may be affected. The leaves are much smaller than those on normal plants of the same age.

PEPPER

Pepper (*Capsicum annuum*), when inoculated with the lima bean virus, does not always give consistent or uniform symptoms. A distinct retardation of growth is the first evidence of infection and is noticeable in from 10 to 14 days after inoculation, followed soon thereafter by a slight clearing of the veins. The basal portion of the leaf is more or less uniformly yellowed (pl. 3, *A*), although sometimes yellowish irregularly shaped spots, distributed over the leaf and between the veins, are developed instead. These spots resemble typical mottling, and can be seen to the best advantage by transmitted light. The yellow color of infected leaves gradually increases in intensity with the increased age of the plant. A slight copper color may replace some of the yellow in old leaves. In the late stages of the disease, the veinlets are perceptibly cleared, giving the leaf somewhat the appearance of network.

TOMATO

The tomato (*Lycopersicon esculentum*) plant is badly stunted and malformed when infected by the lima bean virus (pl. 3, *D*). The mosaic symptoms appear in about 10 days after inoculation. The leaves are mottled, and the new leaf and stem growth is decidedly filiform. The plants frequently die.

CUCUMBER

Young cucumber plants (*Cucumis sativus*), when inoculated with the lima bean virus, show symptoms in about 6 to 10 days. Growth is retarded, and there is a decided clearing of the veins of the leaves.

The leaves are yellowed, the margins curl slightly upward, and as the plant becomes older a glossy appearance develops on the stem from the leaves to the roots. Plants so affected die within a few days. The symptoms are very similar to if not identical with those caused by the cucumber virus.

ZINNIA

The lima bean virus on zinnia (*Zinnia elegans*) does not cause much if any stunting of the plant. The symptoms appear in from 5 to 10 days after inoculation. Soon thereafter mottling occurs on the leaves above the inoculated pair, the spots becoming more distinct with the increasing age of the plant. In the late stages of development, the light and dark spots are sharply defined. The apex of the leaf in most cases becomes uniformly yellowed and very often growth of that part of the leaf is retarded, causing some malformation (pl. 3, *F*). Mottling occurs on the petals and on the leaves of branches developed below the inoculated leaves.

POKEWEED

Pokeweed (*Phytolacca americana*), like zinnia, is little injured by the lima bean virus. In about 2 weeks after inoculation light-yellow spots (pl. 3, *C*) appear scattered irregularly over the surface of the leaf. These spots have no definite outline and bear no order or arrangement in relation to the midrib and veins of the leaf.

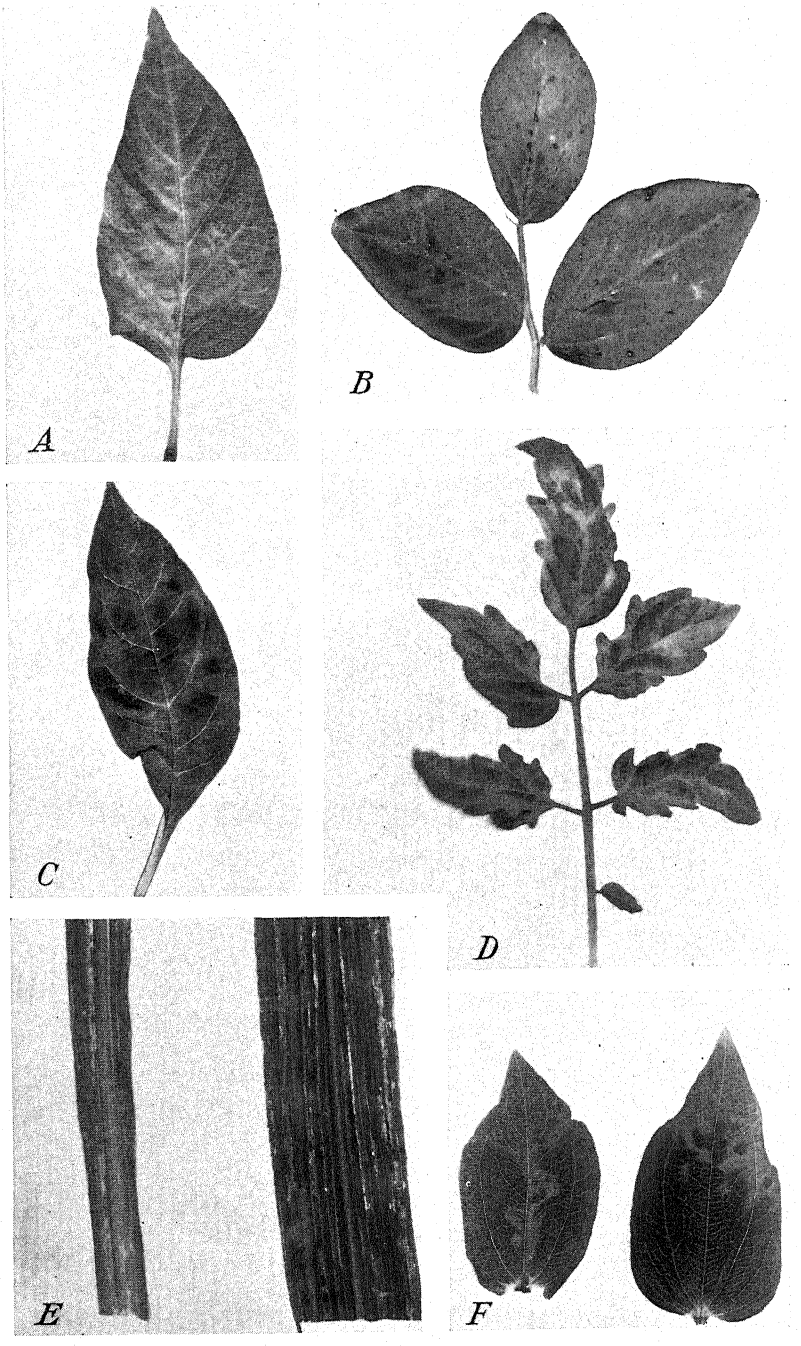
BROADBEAN

The broadbean (*Vicia faba*), although susceptible to the lima bean virus, has never given any consistent results in inoculation tests. Sometimes typical local lesions are produced, and occasionally systemic infection has been obtained. If the inoculum is taken directly from the lima bean plant, no local lesions, or only a very few, develop. If, however, the inoculum is derived from the Turkish tobacco, typical local lesions (pl. 3, *B*) appear in about 4 or 5 days. These lesions are somewhat purplish, and while not so numerous as those on cowpea, they closely resemble them. What the difference in the viruses consists in is not certain, but it has been suggested that the virus is more potent when taken from tobacco than when derived from lima bean.

Systemic infection occurs in a very small percentage of inoculations. The leaves are mottled, but little if any dwarfing of the plant results. When inoculated back to lima beans, the normal lima bean mosaic symptoms are always obtained.

CORN

Wellman (*11*) showed that many plants of the Gramineae, among them corn (*Zea mays*), were readily infected when inoculated with the celery mosaic virus. Price (*9*) proved later that the cucumber virus would also infect corn. The writer has inoculated *Zea mays* with the lima bean virus and has obtained only very mild symptoms in the form of faint streaks characterized by a clearing of the veins. While the infection becomes systemic, no injury to the plant or retardation of growth results. If, on the other hand, popcorn (*Z. mays everta*) is inoculated with the same virus very different results are obtained. In about 7 days after inoculation some yellowing of the leaves occurs, but the most striking symptoms are long alternate green and yellow



Symptoms of lima bean mosaic on various hosts: *A*, Vein clearing and yellowing of leaves of *Capsicum annuum;* *B*, local lesions on leaves of *Vicia faba;* *C*, mottling of leaves of *Phytolacca americana;* *D*, malformation of leaf of *Lycopersicon esculentum;* *E*, yellow streaks and spots on leaves of *Zea mays everta* (popcorn); *F*, leaves of *Zinnia elegans* showing yellow color and retarded growth of apical ends.

streaks (pl. 3, *E*) on the leaves. Later, islands of yellow tissue develop outside or between the vascular bundles. These partly chlorotic areas vary greatly in size and form. The disease increases in severity with the increase in age of the plant. The symptoms become more conspicuous with each new leaf developed until finally almost the entire leaf is uniformly yellowed. In the later stages of the most severe cases the margins of the leaves and the tips of young leaves die and turn brown.

VARIETAL RESISTANCE AND SUSCEPTIBILITY

No attempt has been made to collect all the different varieties of lima beans. Those that have been included in the experiments were obtained from several seed companies. The question of synonymy has not been taken into account, each seed lot being carried under the name given it by the contributor.

The results of identical inoculations shown in the following tabulation indicate that the small-seeded sieva types are susceptible to the lima bean virus, whereas the large-seeded Fordhook sorts are resistant. There seems to be no correlation of pole and bush varieties with susceptibility and resistance.

Variety:	*Reaction*[1]	Variety—Continued	*Reaction*[1]
Burpee Best	−	Hopi	+
Burpee Improved	−	Jackson Wonder	+
Carpenteria	−	King of the Garden	−
Challenger	−	Large White	−
Detroit Mammoth	−	Leviathan	−
Dreer Bush	−	McCrea	−
Dwarf Large White	−	New Wonder	−
Early Jersey	−	Seibert	−
Florida Butter (pole)	+	Sieva	+
Florida Speckled	+	Willow Leaf	+
Fordhook	−	Woods Prolific	+
Henderson Bush	+		

[1] Minus signs (−) indicate resistance; plus signs (+), susceptibility.

TRANSMISSION

SEED

There is no evidence that the lima bean mosaic is carried in the seed. In 1935, seed collected from mosaic plants in the field were planted and allowed to grow until the third trifoliate leaf was fully developed. Of 892 plants from this seed that were carefully examined, none had developed mosaic.

INSECTS

The fact that insects transmit mosaic viruses from one plant to another is so well known that it was not thought necessary to go extensively into this phase of the problem. To demonstrate that the lima bean mosaic is no exception, however, a few experiments were conducted in which two species of aphids, *Aphis gossypii* Glov. and *Myzus persicae* (Sulz.),[6] were employed.

The technique was that usually employed in studies of this kind and briefly was as follows: The aphids were reared in cages, *Aphis gossypii* on cucumbers and *Myzus persicae* on pepper plants. When needed,

[6] These two colonies of aphids were each descended from a single individual and were reared in cages to prevent mixing. The identifications were made by P. W. Mason, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

a dozen or more individuals were transferred to mosaic lima bean plants and allowed to feed for about 24 hours. At the end of that time, young lima bean plants were introduced into the cage and the diseased plants cut down with the expectation that the insects would crawl to the healthy plants and feed on them.

The results were so striking as to leave no doubt of the ability of these insects to carry the virus. The symptoms produced were typical of lima bean mosaic, and the damage was even more severe than when the inoculations were made mechanically by rubbing the juice of mosaic-infected plants into the leaves of healthy ones. At the end of 4 days the symptoms appeared, and in 5 days the terminal bud and young trifoliate leaves were badly malformed. Usually some of the plants died a few days later. The experiments with these two species of aphids were repeated several times with identical results. The objective of the experiment having been attained by demonstrating that insects may transmit the disease, no other insects were tried. It is not improbable that the same results might be accomplished with other species of aphids and even with other kinds of insects.

PROPERTIES OF THE VIRUS

The physical properties of the virus studied were (1) aging in vitro, (2) thermal inactivation, and (3) effect of dilution in water. These properties were selected because it was believed that they would be useful in the identification of the virus or in demonstrating its relationship to other viruses to which it is apparently closely related.

AGING IN VITRO

Aging of the expressed juice of mosaic-infected plants may be useful in identifying a virus, if the experiments are carried out under known control conditions. These conditions should duplicate as nearly as possible the ones originally employed in studying the virus with which the virus under investigation is to be compared. Unfortunately no fixed standard has been followed by all investigators. Johnson and Hoggan (*5*) recommended a temperature of 22° C., and that temperature has been employed. The leaves of lima bean plants infected with mosaic were ground to a fine pulp, and the juice was extracted by squeezing it through cheesecloth. The undiluted juice was then stored in a stoppered Erlenmeyer flask in the dark in an incubator held at a constant temperature (22° C.). Preliminary experiments showed that under these conditions the juice was viruliferous for more than 24 hours, and no shorter period of time was tried. At the end of each 24 hours, for a period of 12 days, a small quantity of the juice was removed from the flask, diluted with about 2 parts of water, and used to inoculate 10 small tobacco plants. In order to avoid any possibility of introducing the virus of other plants, as for example, tobacco, a separate dissecting needle wrapped in absorbent cotton was used for each plant. Symptoms appeared in about 5 days on plants inoculated with extracts that had been aged for the first 3 to 4 days. With extracts aged longer than 4 days, the time between inoculation and the appearance of the first symptoms gradually increased with the increase in the length of the aging period. The results of two separate experiments are shown in table 1.

TABLE 1.—*Results of aging lima bean virus extract at 22° C. on infectivity*

Period of aging (days)	Plants inoculated	Plants infected	Period of aging (days)	Plants inoculated	Plants infected	Period of aging (days)	Plants inoculated	Plants infected
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
0	30	30	5	20	20	8	20	1
1	20	20	6	20	13	9	20	0
2	20	20	7	20	12	10	20	0
3	20	20						

THERMAL INACTIVATION

In testing the lima bean virus to determine the temperature producing inactivation, the usual method of subjecting 2 cc of an undiluted plant extract in a glass tube to a constant temperature in a water bath for 10 minutes was employed. At the end of the heating period the extract was rapidly cooled and was then diluted with about 2 volumes of water. Higher dilutions were not employed, since it was shown by McKinney (7) that dilutions of 1 to 100 or more reduced the potency of some virus extracts. The extract was obtained from lima bean plants. The combined results of several experiments are shown in table 2.

TABLE 2.—*Results of heating lima bean mosaic virus extract at different temperatures on infectivity*

Temperature (° C.)	Plants inoculated	Plants infected	Temperature (° C.)	Plants inoculated	Plants infected	Temperature (° C.)	Plants inoculated	Plants infected
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
50	20	10	65	40	25	75	30	0
55	20	13	67.5	30	10	Not heated (controls)	30	30
60	30	22	70	30	0			

EFFECT OF DILUTION

The extract was obtained from the leaves of lima bean plants, and the dilution was made with hydrant water. The combined results of several different experiments are shown in table 3.

TABLE 3.—*Results from diluting lima bean virus extract on infectivity*

Dilution	Plants inoculated	Plants infected	Dilution	Plants inoculated	Plants infected	Dilution	Plants inoculated	Plants infected
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
1:100	30	25	1:2,000	40	17	1:10,000	20	3
1:200	20	16	1:3,000	30	17	1:20,000	20	0
1:500	30	23	1:5,000	30	12	No dilution (controls)	40	35
1:1,000	40	26						

DISCUSSION

The facts presented thus far appear sufficient to warrant the conclusion that there is a destructive and distinctive virus disease that produces characteristic symptoms in certain varieties of lima beans. It has also been suggested that the causal virus is similar to or identical with the cucumber virus of Doolittle (3) (cucumber virus 1 of Johnson).

It would be logical to assume that the lima bean virus might be a member of the group of legume viruses studied by Zaumeyer and Wade (*13*), but in view of the host range shown (table 1) this appears not to be the case.

The results of these investigations show that the lima bean virus produces symptoms similar to those caused by the cucumber and celery viruses on a number of different hosts. The host range of the celery virus has been extensively studied by Wellman (*12*) and was thought by him to be different from that of the cucumber mosaic virus. The celery mosaic virus, however, was later shown by Price (*9*) to be very similar to the cucumber virus and is considered by him to be no more than a strain of the latter.

The lima bean virus is, without much doubt, very similar to the cucumber mosaic virus and the celery virus. It can, therefore, be identified as a member of the cucumber virus group. Because it differs in some respects from the cucumber virus, it remains to be determined whether these differences are sufficient to justify classifying it as a strain of the cucumber virus. It causes a type of primary lesion on the inoculated leaves of tobacco plants (pl. 2, *D*) not found on other leaves of the plants and, so far as the author is aware, such lesions do not occur on leaves of tobacco plants inoculated with the virus from cucumber or celery.

Perhaps the most significant difference between the mosaic of lima bean and that of celery and cucumber is that the lima bean virus will infect *Vicia faba* while the viruses of celery and cucumber do not. No reference has been found to results showing that the celery and cucumber viruses will cause any type of infection on the horsebean. On the other hand, local and systemic infections are obtained when the broadbean is inoculated with the lima bean virus. Although the percentages of local and systemic infections on the broadbean are comparatively low, the symptoms are clear and convincing and back-inoculations on tobacco or lima bean have yielded positive results. These differences seem to support the conclusion that there is a possible strain difference between the cucumber and lima bean viruses.

Wellman (*10*) showed that a temperature of 75° C. for 10 minutes was necessary to inactivate the celery virus. In several experiments conducted by the writer, the lima bean virus was inactivated at 70°, and only a little more than 30 percent of the plants inoculated with extract that had been heated to 67.5° for 10 minutes were infected.

The gradual inactivation of plant viruses on aging in vitro has been regarded by some investigators as significant and useful in their classification. The writer has shown (table 1) that the lima bean virus retained its full potency for 5 days when stored at 22° C. The potency was somewhat reduced in 6 and 7 days and the virus was completely inactivated in 9 days. Only 1 plant out of a total of 20 in two experiments developed symptoms after the extract was aged for 8 days. It was shown by Wellman that the celery virus withstood aging from 6 to 8 days at 18° to 20°, which might be expected because of the lower temperature used. The cucumber virus withstands even less aging, being usually inactivated in 3 to 4 days. The ability of the lima bean virus to withstand longer aging than the cucumber virus would seem to indicate also that it is probably somewhat different from the cucumber virus.

Except for those mentioned, only slight differences can be detected in symptomatology. The cucumber and lima bean viruses have been compared on a number of different hosts and in general they agree closely. The lima bean and cucumber viruses both cause filiformities to the leaves of tomato, while the celery virus does not, according to the work of Wellman (*10*).

Wellman was unable to obtain local lesions or systemic infection of the Black cowpea. In a parallel experiment the writer inoculated the Black cowpea by rubbing the upper surface of the leaf with viruliferous juice of the celery, cucumber, and lima bean viruses, and obtained numerous local lesions in all cases. There appeared to be some slight differences in the characteristics of the lesions but it is not believed that they were sufficiently distinct to signify a difference in symptomatology.

The results of these investigations indicate that the lima bean mosaic is caused by a virus very similar to that which causes cucumber mosaic, but not identical with it. The slight differences observed in symptoms of the suscepts are not believed sufficient to justify designating the causal virus as new and distinct but as a strain of the cucumber virus. The writer would suggest designating it as the lima bean mosaic strain of the cucumber virus.

SUMMARY

A mosaic disease of lima beans, similar to the mosaic of cucumber and celery, is described. The disease differs slightly in symptomatology on several different hosts.

The following varieties of lima beans were found to be susceptible: Jackson Wonder, Hopi, Florida Speckled, Florida Butter, Henderson Bush, Woods Prolific, Willow Leaf, and Sieva. The resistant varieties were Burpee Best, Burpee Improved, Carpenteria, Challenger, Detroit Mammoth, Dwarf Large White, Dreer Bush, Early Jersey, Fordhook, King of the Garden, Leviathan, Large White, McCrea, New Wonder, and Seibert.

The lima bean virus differs slightly from the cucumber virus in symptomatology and in some of its physical properties, such as aging in vitro and inactivation upon heating. However, it does not differ enough from the cucumber virus to be classed as distinct. It is proposed, therefore, that it be designated as the lima bean mosaic strain of the cucumber virus.

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SAND DUNES OF RECENT ORIGIN IN THE SOUTHERN GREAT PLAINS[1]

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INTRODUCTION

In recent years, as a result of cultivation, grazing, and drought, sand dunes have developed on large areas of land throughout the southern Great Plains, especially between the Canadian and Arkansas Rivers. Data secured from an aerial survey also show many such sites scattered through Colorado, Kansas, Nebraska, Wyoming, North Dakota, and South Dakota. Their combined extent is approximately 15,000 acres.

Considerable effort has been expended to control and reclaim sand-dune areas throughout the world—in Palestine, Australia, Germany, France, the United States, and many other countries. The usual treatment is to prevent sand movement through stabilization by means of vegetation (*4*).[3] Often barriers are constructed by artificial means to serve as protection against drifting sand. The relationships between sand dunes and vegetation have been discussed by a number of workers, among whom are Cowles (*2*), Harris (*5, pp. 41–46*), and Rempel (*7*).

The physical characteristics of dunes, their formation, shape, size, and movement have been described by Cornish (*1*), Cowles (*2*), King (*6*), Rempel (*7*), and others. Studies of sand movement and field operations to control it have been conducted on coastal, river, and lake dunes where there is a constant supply of sand. So far as can be determined, however, no experimental studies have been made of sand-dune areas in the Great Plains.

Three types of sand dunes are found in the Great Plains. The first and least extensive is the "blow-out" type, which develops around wells or from roads and cattle trails. The second type, with which we are here concerned, develops as a result of the destruction of surface cover. Both of these types as a rule are bare of vegetation and are of recent origin. The third type is formed by wind action on outwash material and is usually fairly well stabilized by native vegetation.

Sand dunes of recent origin are often found near older dunes, which indicates that in times past wind action has spread the outwash considerable distances, though not always in dune form.

Studies dealing with the origin and character of the second type of dune areas and with methods of stabilizing and utilizing them were begun in January 1936 by the Soil Conservation Service near Dalhart,

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[2] Acknowledgment is made to several workers of the Soil Conservation Service in Region 6 for their assistance in making measurements and for their suggestions and interest in the problems. Thanks are given especially to B. H. Hopkins, project manager, Dalhart, Tex., and to J. C. Wilcox, Amarillo, Tex., for their aid and encouragement.

[3] Reference is made by number (italic) to Literature Cited, p. 917.

Dallam County, Tex. Two thousand acres of land were obtained for study, 912 of which were leased. The remainder was obtained through cooperative agreement. The 2,000-acre area fell naturally into three distinct dune plots. After measurements were taken, two plots were found to be strikingly similar (table 1). One of these, plot 1, was used as a work plot, and the other, plot 2, as a check. The third plot was in an earlier stage of dune formation, and the study of dunes on this plot is not reported in this paper.

TABLE 1.—*Slope, land cover, soils, and erosion on dune plots 1 and 2 near Dalhart, Tex., Apr. 23, 1936*

Item	Plot 1	Plot 2
Slope:	*Percent*	*Percent*
0 to 2 percent	83	90
2 to 8 percent	11	2
8 to 12 percent	6	8
Land cover:		
Abandoned land	58	54
Pasture; one-third of stand killed	8	1
Pasture; one-third to two-thirds of stand killed	7	6
Pasture; over two-thirds of stand killed	27	39
Soils and subsoils:		
Soils:		
Amarillo loam	1	--------
Amarillo fine sandy loam	20	32
Amarillo loamy fine sand	21	19
Subsoils:		
Clay	1	4
Clay (moderately light)	10	--------
Sandy clay loam	12	13
Sandy clay loam (light)	1	4
Loamy fine sand	--------	9
Sandy clay loam	14	3
Dune sand	20	16
Erosion:		
Accumulation:		
Moderate, 6 to 12 inches deep	(1)	2
Moderate, 6 to 12 inches deep (hummocky)	(1)	3
Erosion—Continued.		
Accumulation—Continued.	*Percent*	*Percent*
Severe, 12 to 36 inches deep	7	8
Small dunes, 36 to 72 inches high	2	2
Large dunes, over 72 inches high	6	8
Removal:		
Moderate, 25 to 75 percent of the A horizon removed	(1)	--------
Severe, A and upper B horizons removed	22	20
Very severe; lower B horizon, the C horizon, or the parent material, removed	14	6
Accumulation and removal:		
Shallow accumulation, 0 to 6 inches, slight removal	16	6
Moderate accumulation, slight removal	3	4
Shallow accumulation, moderate removal	5	8
Moderate accumulation, moderate removal	12	17
Severe accumulation, moderate removal	5	9
Small dunes, moderate removal	5	--------
Moderate accumulation, very severe removal	--------	7

[1] Less than 1 percent.

SAND DUNES ON THE STUDY PLOT

The sand dunes of plot 1 like those of the check plot are on land that had not been plowed before the formation of the dunes. They developed as a result of wind action on an 80-acre field that lies to the southwest of the plots. This field was cultivated from 1907 to 1914 and was then used for grazing. Dry years and heavy grazing of the field prevented growth of sufficient vegetation to hold the soil. According to the best information obtainable,[4] it was in 1926 that small hummocks of sand were first noticed to be forming against fence rows and piling against buildings, and unpalatable plants (Russian-thistle and soapweed) began to appear.

After 1929 striking growth of the dunes was noticed. Instead of forming on the original broken sod, however, the dunes formed to the north and east, since the prevailing winds are from the southwest. As years passed the large dunes shifted farther north and east, leaving large, completely denuded areas behind (fig. 1). The coalescence of

[4] From W. H. Lathem, Land Office, Dalhart, Tex.

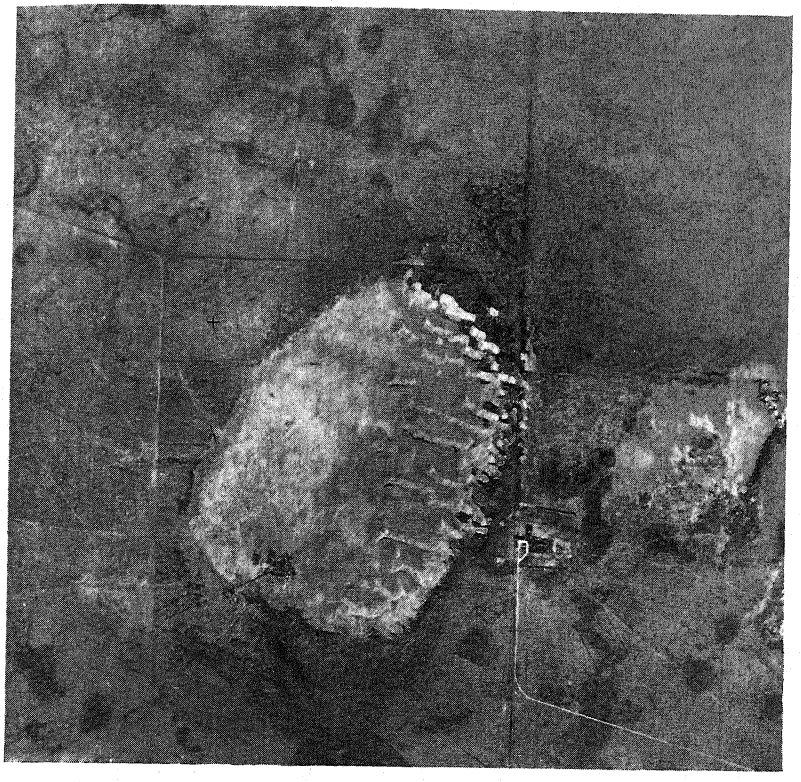


FIGURE 1.—An airplane view of a typical dune site, October 1936. The sand dunes are east and north of the denuded area.

dunes caused a great increase in their height as well as in their length and width.

Usually the dunes have long, gently rising windward slopes and crescent-shaped leeward slopes (fig. 2). Dunes as low as 6 feet may

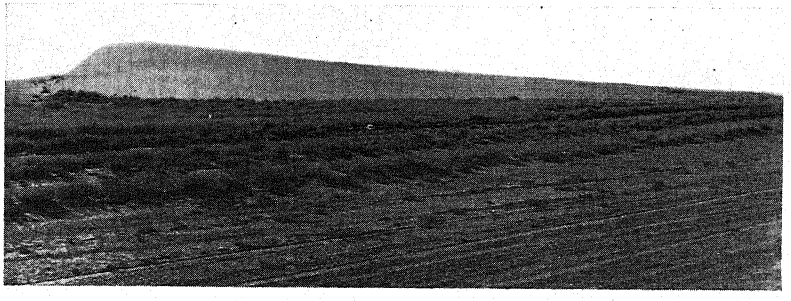


FIGURE 2.—A typical sand dune in the background. The windward slope is long and gently rising; the leeward slope is much more abrupt and generally crescent-shaped.

take this form and may continue to build up to greater heights when weeds become lodged on them or the packing of the sand prevents it from blowing. There is some evidence that crescentic sand dunes form most readily on hard, level substratum. There is also evidence

that the crescent shape of the leeward side accounts for the increase in size of the dune. When the wind approaches the top of the dune it drops its load of sand as a result of the formation of eddies or wind currents that move at right angles to the prevailing winds.

ORIGINAL AND PRESENT VEGETATION

Originally, the entire study plot was probably dominated by a mixed type of vegetation. Blue grama (*Bouteloua gracilis* (H. B. K.) Lag.) and buffalo grass (*Buchloë dactyloides* (Nutt.) Engelm.) were undoubtedly the conspicuous species on the gently rolling slopes, and side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.) and silver beardgrass (*Andropogon saccharoides* Sw.) were found in depressions. Red three-awn (*Aristida longiseta* Steud.) and sand dropseed (*Sporobolus cryptandrus* (Torr.) A. Gray) were species on immature soils and in disturbed places. If plants of sand sage (*Artemisia filifolia* Torr.) and *Yucca glauca* Nutt. were present, they were probably few.

The conspicuous grass at present is blue grama, but relict plants of buffalo grass and the mid and tall grasses occur over the area.

THE SAND-DUNE COMPLEX ON PLOTS 1 AND 2

It must be kept in mind that plots 1 and 2 were similar in practically every respect to begin with, and that plot 1 is the plot on which the dunes were stabilized and plot 2 is the check plot. On both of the plots a typical sand-dune complex consists of four distinct divisions: (1) The sand dunes; (2) eroded land, which occurs to the west of and between the dunes; (3) surrounding vegetated land; and (4) a border area that separates the sand dunes and eroded land from the adjacent less affected portion.

THE SAND DUNES

The sand dunes range from 50 to 770 yards in length and are generally 30 to 50 yards wide (fig. 3). Their height depends, appar-

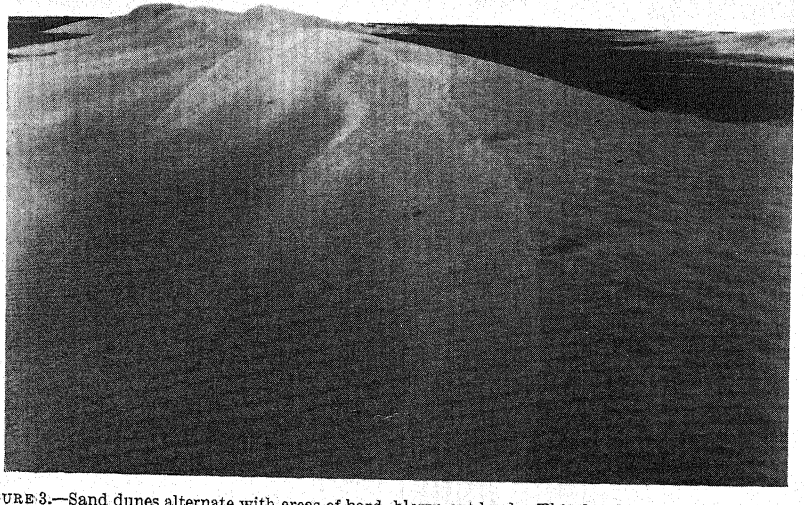


FIGURE 3.—Sand dunes alternate with areas of hard, blown-out land. This dune is approximately 22 feet in height.

ently, on their age and the direction and velocity of the wind. Data regarding dunes on plot 1 in 1935–36 are given in tables 2 and 3. The height of the highest dune measured in 1936 was 26 feet (table 3). The axis of most of the dunes is east and west, but in some it may be a little southeast and northwest, owing to the direction of the prevailing wind. The dunes are irregularly spaced, the distance between them ranging from 50 to 400 yards. They are generally compact beneath and moist a few inches below the surface.

TABLE 2.—*Number of dunes, classified as to length and width, on plot 1, 1935–36*

Dimensions and year	0–100 feet	101–300 feet	301–500 feet	501–700 feet	701–900 feet	901–1,100 feet	1,101–1,600 feet	1,601–2,400 feet	Total dunes
Length:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1935	3	19	14	6	7	5	0	0	54
1936	0	5	1	3	2	0	2	1	14
Width:									
1935	17	37	0	0	0	0	0	0	54
1936	2	6	6	0	0	0	0	0	14

TABLE 3.—*Height of 6 dunes on plot 1, 1935–36* [1]

Year	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
	Feet	*Feet*	*Feet*	*Feet*	*Feet*	*Feet*
1935	12	12	14	14	15	18
1936	21	24	18	25	26	21

[1] Height of all dunes ranged from 1 to 19 feet in 1935, and from 2 to 26 feet in 1936.

THE SUBSTRATUM

The substratum on which the dunes rest, as well as that between and around them, is very compact, hard, and fairly level. It consists of the subsoils of uncultivated land that has been eroded to various depths, in some places to as much as 4 feet. The fact that the prevailing winds are from the southwest accounts for there being large areas of hard substratum to the west and south of the dunes and accumulations of sand from 6 inches to 2 feet in depth to the north and east. In places this hard land assumes the appearance of desert pavement, owing to the exposure of caliche and other fragments too heavy to be moved by the wind.

THE CRITICAL AREA

The critical area is an ecotone or border strip that lies between the range grassland and the eroded substratum and dune lands. This border is designated "critical area" because of its hummocked condition and the presence of piles of loose sand devoid of vegetation that make it a possible source of materials for dune development, and because it possesses other characteristics that apparently aid in dune formation, such as vulnerability to the winds that sweep unhindered across the hard land, picking up material as they go.

THE MOVEMENT OF DUNES

Small dunes or mounds coalesce to form large dunes. In June 1935, according to a survey of the Operations Division of the Soil Conservation Service project at Dalhart, Tex., there were on plot 1

some 50 small dunes from 2 to 18 feet in height and of various lengths and widths. Although some were merging, most of them were distinct. In June 1936 on the same plot there were only five relatively large dunes and nine outlying smaller ones (table 2).

When dunes are small, their rate of movement is very rapid. Measurements over a 2-week period showed that one dune moved as much as 26 feet. Most of this movement undoubtedly occurred in the course of the one storm lasting several hours that took place during this period.

When dunes reach a height of more than 16 feet, their movement is less rapid. Measurements of the larger dunes, however, showed that they had progressed in a northeast direction an average of 37 feet in 1 year, the sand being rapidly removed from the west end of the dunes and added to other dunes to the north and east.

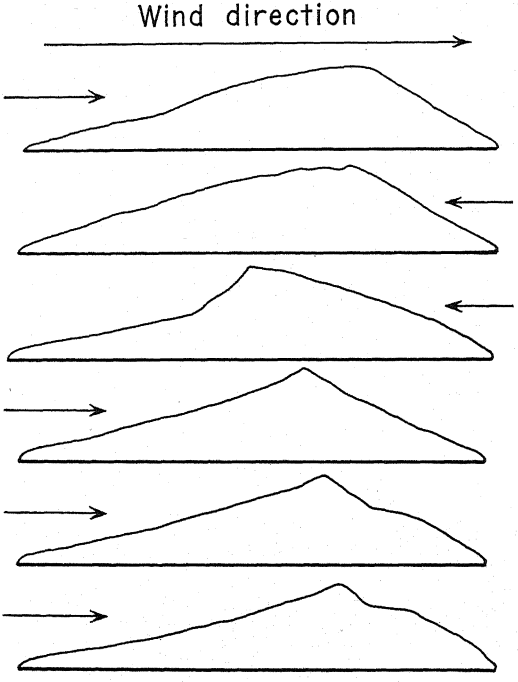


FIGURE 4.—Effect of reversal of wind on cross section of a dune. (After King (6).)

The rate of wind movement is one of the most influential factors in the moving of sand, but this force is definitely modified by local conditions. The greatest shifting of materials occurs during the spring blowing season and when wind velocities are above 20 miles per hour. As a rule, the higher the velocity of the wind the greater the movement of the sand; but when the land is in condition to blow, as it often is in February, March, and April, winds of low intensities move large quantities of material.

Since the prevailing direction of the wind is from the southwest, the dunes are moving in a northeast direction. Severe windstorms, however, come out of the north and west, and although they fail to change the location of the larger dunes, the slopes may be completely reversed, the steep leeward slope facing southwest and the windward slope facing northeast (fig. 4).

STABILIZATION OF DUNES

Since large machinery is expensive and is not available to all farmers and ranchers, it was necessary to develop economical as well as efficient methods by which the sand-dune areas could be stabilized. One practical solution lay in the utilization of wind power to redistribute the materials that this same force had built up into dunes. This principle has been employed in practically all the work.

METHODS OF DECREASING HEIGHT OF DUNES

In order to decrease the height of dunes sufficiently to permit their being effectively planted, which is necessary for stabilization, four means of facilitating movement were employed, namely, (1) wind intensifiers, (2) drag-pole, (3) one-way disk, and (4) tractor and blade.

Wind Intensifiers

Three types of intensifiers were tried, the signboard (fig. 5), the gunny sack, and the wind channel. The signboard type was constructed by nailing galvanized iron or boards between posts at different heights above the sand level on the crest of the dune. Gunny sacks were filled with sand and put at different spacings on top of the dune. Wind channels were dug in the dune; these were generally 3 feet wide and 4 feet deep.

The wind intensifiers were placed at the crest of the dune. They proved very efficient in moving sand; large gaps 4, 5, and 6 feet deep

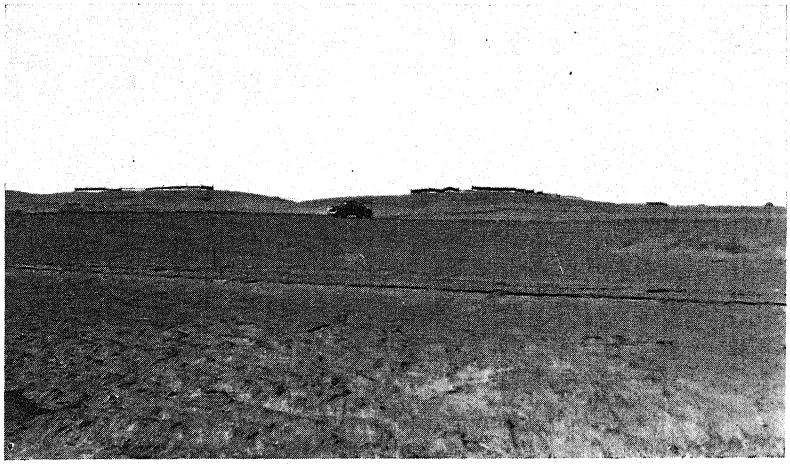


FIGURE 5.—Signboard type of intensifier made of boards and galvanized iron and placed on the crest of the dune.

were dug out and the sand was carried out beyond the crest. The sandbags were most effective, since they would lower as the wind removed the sand from around them. During one storm, a dune was lowered about 2 feet by the use of sandbags. The crest of the dune flattened down, and the dune itself moved forward 6 to 10 feet. The use of wind intensifiers in lowering dunes did not prove to be practical, however, because too much manual labor was required both in setting them up and in digging out those that had been undermined and had toppled over or were totally or partly covered by the moving sand during heavy windstorms.

Drag Pole

A second means of destroying the steep slope to the leeward side of the dunes, and thus allowing the wind to carry the sand out beyond, is the use of a drag pole. The drag pole is an 8- by 8-inch timber of

sufficient length (for the largest dunes, 20 feet). One or two horses are hitched to each end of the pole and it is dragged along the sharp edge of the dune at right angles to the crest. Attaching a disk at the top aids in breaking down the steep slope.

The drag pole can be used more advantageously than the wind intensifiers because it requires less hand labor and a greater area can be covered in a shorter period of time. By this means, as by the use of the intensifiers, the wind is prevented from forming eddies and as a result it carries huge quantities of sand out beyond the dune. The dune seems to move en masse when the crest is broken down by this method. One dune on which the drag pole was used was lowered 15 feet in 6 months.

Of the four methods here suggested for decreasing the height of dunes, the use of the drag pole is the most efficient as well as the most economical. Furthermore, as this method does not require the use of expensive machinery, it is practicable for the farmer and rancher.

One-Way Disk

The sandy material making up a dune often becomes compacted, owing to vegetative growth, trampling of stock, and rain. The best means yet found to loosen this sand so that it can be moved by wind action is a one-way disk plow. Disk-harrowing the dunes with teams is economical as well as effective.

Tractor and Blade

Another method employed to spread the sand is the use of a tractor and blade. By this means dunes can be flattened and leveled, and when it is supplemented by wind action, quick results are obtained. One dune worked in this way was lowered from 20 feet to 5 feet in 6 months and at the end of that time was in condition to be planted (fig. 6).

The procedure with the blade is to make one to three turns over the highest points of the sand dune, after which the equipment is moved to another dune which is treated similarly. This treatment gives the wind a chance to move the loose sand. From 6 to 12 of these treatments are generally necessary to affect materially the height of the dune.

Listing

One method employed to prevent the sand's moving from one dune to accumulate on another is that of listing the hard land around and between the dunes (fig. 6, *A*). This procedure prevents more sand from accumulating on the dune and also catches the material blown off the dune. The need for deep listing cannot be overemphasized. Shallow-listed areas have continued to blow, whereas the deep-listed lands on the experimental areas have not blown. Relisting has been done over much of the area, and the soil, even that which was badly eroded, has been mixed with sand and other wind-blown material to such an extent that it is probable that a crop can be produced on most of it.

Stabilization through Vegetation

The development of a vegetative cover, whether cultivated crop or native vegetation, is necessary for complete stabilization.

The critical area between the hard eroded land of the study plot and the native pasture was a mass of loose, drifting sand, which was often hummocked by the Russian-thistle that grew there. Listing this area and planting it to grain sorghums held the sand and prevented it from

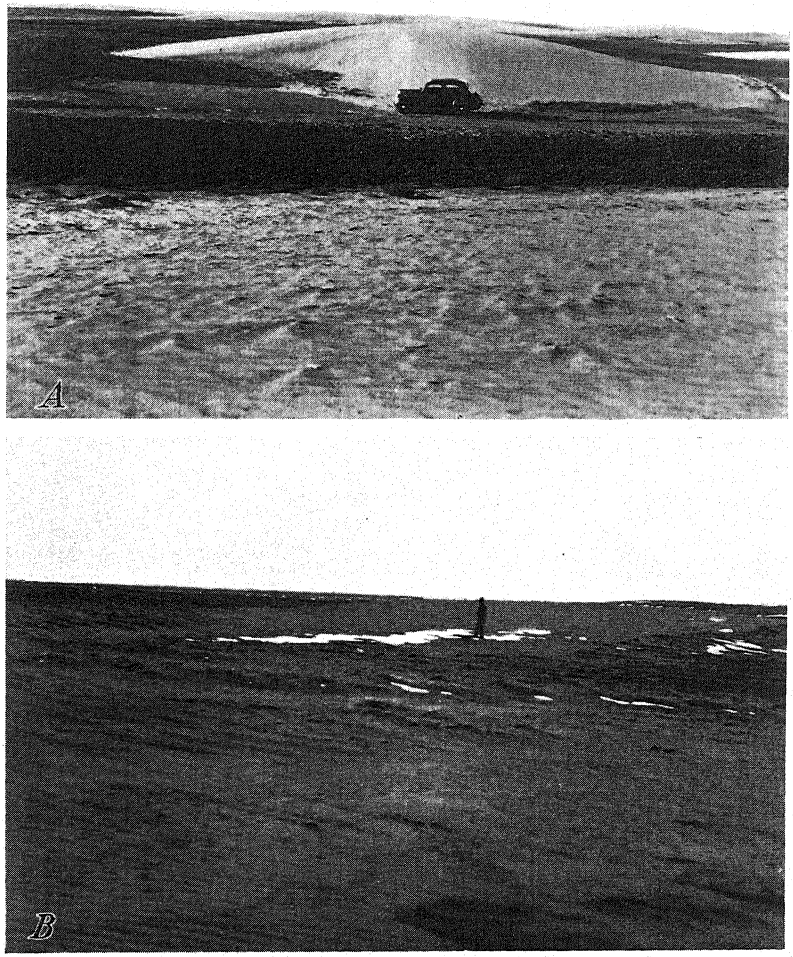


FIGURE 6.—*A*, An isolated dune with the typical half-moon shape. The hard land around this dune has been listed. *B*, The man is standing approximately in the place occupied by the car in *A*. This dune has practically coalesced with the one to the north. It has been lowered approximately 15 feet.

being blown across the hard land to cause further erosion and accumulation.

As a result of listing the hard eroded lands and cutting off some of the mechanical action of the wind by treating the critical area, vegetation developed on many of the areas that were formerly bare. Although this cover was mainly Russian-thistle, it was sufficient to

prevent blowing and even to catch and hold the moving sand. On the check plot no weeds appeared on the bare land.

The fine sandy soils composing the dunes, when properly cultivated and supplied with sufficient moisture, are capable of producing the abundant vegetation that is essential to permanent stabilization. After the dunes had been lowered by mechanical means to a height that permitted their being effectively planted, various row-crop species, Sudan grass, milo, kafir, and hegari, were drilled and listed over and around the dunes, and over most of the area a sufficient stand was produced to protect the soil adequately (fig. 7).



FIGURE 7.—Site in figure 5, about 14 months later. The original view was taken from the ground; this picture was taken from the top of an automobile. Good stands of Sudan grass, black amber cane, and kafir have grown on this once badly eroded area. Both the hard substratum and the sand dunes now have a cover adequate to protect the soil from further erosion during the coming year.

Hay of sand reedgrass (*Calamovilfa gigantea* (Nutt.) Scribn. and Merr.) was spread over one dune, 1.1 acres in extent and more than 16 feet high, in June 1936. By July 1937 a dense growth of sand reedgrass and Russian-thistle completely covered this area, the Russian-thistle dominating for the most part on the drier southwest exposure. The grass plants were of good height and formed a relatively thick stand.

SUMMARY AND CONCLUSION

Erosion hazards in the form of sand dunes have developed in recent years on large areas of land throughout the Great Plains. In January 1936, the Soil Conservation Service initiated studies to determine the best methods of stabilizing and utilizing such sites. To accomplish this objective it was necessary to investigate the origin and character of sand dune areas.

As a result of these studies it was found that the sand dunes could be effectively lowered by utilizing the force of the wind to redistribute the materials that this same force had built up into dunes and that

over most of the area a sufficient cover crop could be produced to protect the soil adequately against further erosion.

This study indicates that the better land is capable of producing good crops of grain sorghums if farmed in such a way as to prevent soil drifts (*3*) and also that these dune sites can be returned to grass.

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THE EFFECT OF THE X FACTOR, OF SODIUM CHLORIDE, AND OF THE COMPOSITION OF THE NUTRIENT MEDIA UPON THE GROWTH OF THE FOWL CORYZA BACILLUS, HEMOPHILUS GALLINARUM [1]

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INTRODUCTION

The recent work of Kessens [2] concerning the nature of the growth requirements of the European fowl coryza bacillus indicates the need of the V factor and an additional factor, which he terms the C, or coryza factor, for its growth. Kessens found that the clotted-blood serum of the chicken and pigeon contains X, V, and C factors, and thus meets the requirements for growth, while that of the horse, ox, sheep, goat, rabbit, and cat possess only the X factor and will not support growth. The C factor of chicken serum was destroyed by heating at 90° to 100° C. for 10 minutes. Chicken serum so heated supported the growth of *Haemophilus influenzae,* but it was unsuitable for the coryza bacillus (H. *gallinarum*), although it still possessed the X and V factors. As a result of these studies, Kessens suggests the name *Haemophilus coryzae* for the organism inasmuch as the X factor was not required for growth.

Schalm and Beach [3] report the need of the X and V factors for growth of the American strains of the fowl coryza bacillus which they studied.

The writers [4] observed a considerable difference in the amount of growth that occurred with the coryza bacillus when chicken blood was used at the base of nutrient agar slants and when it was used in the form of a blood-broth medium. The writers [5] also obtained growth with serum from clotted horse blood diluted in the proportion of 1 : 5 with broth at the base of agar slants but not with the same diluted serum broth when used alone.

The experiments herein described were conducted to determine what effects the use of the test-fluid medium at the base of agar slants, as compared to its use alone, would have on the growth requirements of the Rhode Island strains of the fowl coryza bacillus as a result of the difference in growth cited.

[1] Received for publication September 22, 1937, issued June 1938. Contribution No. 510 of the Rhode Island Agricultural Experiment Station.

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[5] Unpublished data.

EXPERIMENTAL DATA

GROWTH FACTORS IN YEAST

Since Thjötta and Avery [6] found that yeast supplies the V factor for *Hemophilus influenzae* yeast extracts and yeast-cell suspensions were prepared to determine their value in supplying the factor necessary for growth of the fowl coryza bacillus. If the organism grew in the yeast media, inoculations for the various tests would be made from them and thus the possibility would be avoided of a carry-over of the growth factors from the blood cultures that otherwise would have to serve as the source of inoculum.

Boiled acidulated yeast extract supplies the V factor. Since Kessens [7] found that the C factor of chicken serum was destroyed by heating for 10 minutes at 90° to 100° C., the question arose as to whether this factor might not have been destroyed in preparing the boiled yeast extract.

Pure dry Fleischmann's irradiated yeast was prepared as follows: 20 g of yeast was added to 100 cc of distilled water and stirred until as much as possible of the yeast was in suspension. The material was then centrifuged and a yellowish, clear, transparent, supernatant extract was obtained. A few drops of phenol red were added to the extract, and sodium hydroxide was used to adjust the pH until it was slightly alkaline to this indicator. The extract was again centrifuged and passed through Seitz filters. The sterile yeast extract was added to the base of nutrient agar slants (Difco dehydrated nutrient agar, 23 g; sodium chloride, 8 g; water, 1,000 cc).

Acidulated yeast was prepared according to the method of Thjötta and Avery,[8] except that the filtrate, after boiling, was made slightly alkaline to phenol red by the addition of sodium hydroxide, then centrifuged, and passed through Seitz filters.

Autoclaved blood extract was prepared according to the method described by Schalm and Beach.[9] The blood of chickens, sheep, goats, and horses was used. Autoclaved blood extract contains only the X factor.

Pure cultures of yeast were used to determine whether suspensions of the yeast might supply some of the growth requirements. Yeast was grown on beerwort agar, and the cells were washed into suspension with sterile physiological saline solution. The pH of the suspension was adjusted to the neutral point as indicated by phenol red.

Two Rhode Island strains of the organism were used for these studies, cultures 44 and 80. Culture 44 had been cultured for over 3 years in the writers' laboratory; culture 80 had only recently been isolated from a field type of coryza.

Growth occurred in the first tubes of yeast extract (unheated) when inoculated from the stock cultures (table 1). Attempts to continue the culture in this type of extract by inoculating from the successful ones, failed after several attempts. However, upon the addition of a loopful of chicken red blood cells to each tube, growth occurred after inoculation, as in the first tubes inoculated from the stock cultures.

[6] THJÖTTA, T., and AVERY, O. T. STUDIES ON BACTERIAL NUTRITION. II. GROWTH ACCESSORY SUBSTANCES IN THE CULTIVATION OF HEMOPHILIC BACILLI. Jour. Expt. Med. 34: 97-114. 1921.

[7] KESSENS, B. H. See footnote 2.

[8] THJÖTTA, T., and AVERY, O. T. See footnote 6.

[9] SCHALM, O. W., and BEACH, J. R. See footnote 3.

The addition of autoclaved blood extract from the chicken, sheep, goat, and horse to the yeast extract also completed the growth requirements (table 1).

The boiled yeast extract plus autoclaved blood extract gave only a poor growth, indicating the destruction of some substance as a result of boiling. Since Kessens [10] called this the C factor, it will be so refered to here.

TABLE 1.—*Growth of the fowl coryza bacillus in yeast extract and in yeast suspension at the base of nutrient agar slants*

Inoculum	Medium	24- and 48-hour microscopic examination [1]		Growth in check medium [2]	
		Culture 44	Culture 80	Culture 44	Culture 80
Cultures 44 and 80 grown in chicken blood.	Yeast extract (unheated)	++	++	++	++
First yeast-extract culture.	do	−	−	−	−
Yeast extract culture	Yeast extract (unheated) and chicken red blood cells.	++	++	++	++
	Yeast extract and autoclaved chicken blood extract.	++	++	++	++
	Yeast extract and autoclaved sheep-blood extract.	++	++	++	++
	Yeast extract and autoclaved goat-blood extract.	++	++	++	++
	Yeast extract and autoclaved horse-blood extract.	++	++	++	++
	Boiled yeast extract and autoclaved chicken blood extract.	±	±	++	++
	Yeast-cell suspension	−	−	−	−
	Yeast-cell suspension plus autoclaved blood extract.	±	±	±	±
	Autoclaved blood extract [3]	−	−	−	−

[1] +=growth; ++=good growth; ±=poor growth; −=no growth.
[2] Check medium consisted of chicken blood at the base of nutrient agar slants.
[3] Chicken, sheep, goat, and horse red blood cells used.

It is evident that yeast extract (unheated) possesses both the V and the C factors, which alone are insufficient for growth. It meets the requirements for growth upon the addition of blood or autoclaved blood extract containing the X factor.

Boiled yeast extract contains the V factor and very little of the C factor, as indicated by the results obtained with this preparation plus autoclaved blood extract. Thus, the presence of the X and V factors do not suffice for good growth of the fowl coryza bacillus. Growth is good, however, in the presence of the X, V, and C factors, as indicated by the use of the unheated yeast extract (line 1, table 1).

The growth obtained from the inoculation of the yeast extract from the stock blood culture can probably be explained on the assumption that sufficient of the X factor was carried over in the inoculum. Thjötta and Avery [11] noted this in their studies of *Hemophilus influenzae*.

The autoclaved blood extract failed to give any growth, showing that other factors, in addition to the X factor are essential for growth (table 1).

Suspensions of the yeast cells plus autoclaved blood extract met the growth requirements. The suspension of yeast cells failed in this respect (table 1).

[10] KESSENS, B. H. See footnote 2.
[11] THJÖTTA, T., and AVERY, O. T. See footnote 6.

GROWTH FACTORS IN CLOTTED-BLOOD SERUM OF HORSE, SHEEP, GOAT, COW, RABBIT, PIG, TURKEY, AND CHICKEN

In each instance the serum employed in these studies was obtained from only one animal of a species, except that of the chicken. The blood was collected in dry, sterile glass bottles and permitted to clot without any agitation. The serum was removed, centrifuged, and passed through Seitz filters for sterilization.

The serum for each test was added to the broth [12] in the proportion of 1:20.

Some of each serum-broth mixture was placed in sterile tubes and some at the base of nutrient agar slants. All were inoculated from cultures maintained in yeast extract plus autoclaved blood extract at the base of nutrient agar slants.

Slides for microscopic examination were prepared from each culture after 24 and 48 hours' incubation at 37° C. After 48 hours' incubation, material from each tube was used to inoculate sterile citrated chicken blood at the base of agar slants to check for growth and for any contamination that might arise.

Growth did not occur in the serum broth tubes of any of the animal serums except those of the chicken and turkey (table 2). The serum broth of each animal at the base of nutrient agar slants showed growth in each instance, as indicated by the fact that the organisms were recovered in citrated chicken blood at the base of nutrient agar slants. Growth was particularly good in the serum of the chicken, turkey, sheep, and goat, and was poor in that of the horse, cow, rabbit, and pig. In the case of the cow and pig serums, growth was evident only at the time of the examination of the chicken blood which had been inoculated from these particular cultures. These results confirm observations previously made by the writers [13] that with chicken blood there is an appreciable difference in the amount of growth obtained in blood broth alone and that obtained in the same material at the base of nutrient agar slants.

The question might arise as to whether the organisms grew or whether they merely survived in certain of the serums inasmuch as recovery of the organisms in chicken blood was used as an indication of growth. In no instance was growth obtained from the serum broth tubes or from the control broths; so it must be concluded either that growth did occur, or that the material at the base of the agar slants was more suitable for the maintenance of the organisms.

In the case of the sheep, goat, chicken, and turkey serums the broth in dilutions of 1:20 at the base of nutrient agar slants was sufficient for continued growth when transfers from the respective tubes were used to inoculate similar ones. Such was not the case with the serums of horse, cow, rabbit, or pig, as growth could not be continued. These tests indicate that the serum of the particular animal employed varied in the concentration of the growth factors when it was used at the base of nutrient agar slants and, except in the case of the chicken and turkey, that it would not support growth when used alone.

Serum from clotted chicken blood diluted 1:20 with broth was boiled 10 minutes, permitted to cool at room temperature, and transferred to sterile tubes and to the base of nutrient agar slants. There was no growth in the heated serum broth in sterile tubes, but in the

[12] Broth, except where the sodium chloride varied in different tests, was prepared as follows: Beef extract, 5 g; peptone, 5 g; salt, 5 g; and water, 1,000 cc.

[13] DELAPLANE, J. P., ERWIN, L. E., and STUART, H. O. See footnote 4.

same material at the base of nutrient agar slants, growth occurred (table 2). Apparently some change took place as a result of boiling, but the fact that growth occurred at the base of the agar slants indicates that the destruction was not complete.

TABLE 2.—*Growth of the fowl coryza bacillus when serum from the clotted blood of various animals was used in broth at the base of nutrient agar slants and when used in broth alone.*

Medium	24- and 48-hour microscopic examination [1]		Growth in check medium [1 2]	
	Culture 44	Culture 80	Culture 44	Culture 80
Horse serum and broth (1:20)	−	−	−	−
Horse serum and broth (1:20) at base of agar slants	±	±	++	++
Cow serum and broth (1:20)	−	−	−	−
Cow serum and broth (1:20) at base of agar slants	−	−	++	++
Sheep serum and broth (1:20)	−	−	−	−
Sheep serum and broth (1:20) at base of agar slants	+	+	++	++
Goat serum and broth (1:20)	−	−	−	−
Goat serum and broth (1:20) at base of agar slants	++	++	++	++
Rabbit serum and broth (1:20)	−	−	−	−
Rabbit serum and broth (1:20) at base of agar slants	±	±	++	++
Pig serum and broth (1:20)	−	−	−	−
Pig serum and broth (1:20) at base of agar slants	−	−	++	++
Turkey serum and broth (1:20)	±	±	++	++
Turkey serum and broth (1:20) at base of agar slants	++	++	++	++
Chicken serum and broth (1:20)	+	+	++	++
Chicken serum and broth (1:20) at base of agar slants	++	++	++	++
Chicken serum and broth (1:20) boiled 10 minutes	−	−	−	−
Chicken serum and broth (1:20) boiled 10 minutes, at base of agar slants	+	+	++	++
Control broth	−	−	−	−
Control broth at base of agar slants	−	−	−	−
Yeast extract and autoclaved red-blood cells	−	−	−	−
Yeast extract and autoclaved red-blood cells at base of agar slants	++	++	++	++

[1] +=growth; ++=good growth; ±=poor growth; −=no growth.
[2] Check medium consisted of chicken blood at the base of nutrient agar slants.

After noting the difference in reaction of the various serums when used with broth alone and when used with broth at the base of agar slants, the writers made another test in which yeast extract plus autoclaved blood extract was used in sterile tubes and at the base of nutrient agar slants. No growth occurred in the sterile tubes (table 2) but at the base of the agar slants growth was good.

THE POSSIBLE ROLE OF AGAR AS USED IN THESE EXPERIMENTS

Since it was apparent that there is a difference between growth of the Rhode Island fowl coryza bacillus in serum broth and in serum broth at the base of agar slants, experiments to determine the nature of these differences were conducted. Since it had also been noted [14] that differences in growth could be detected with different percentages of sodium chloride in the agar when citrated chicken blood was used at the base of the agar slants. Accordingly an experiment was made in which a medium containing 1.5 percent of plain agar (without the addition of nutrients) was compared with a similar medium to which 0.8 percent of sodium chloride was added. The serum of sheep and goat diluted 1:20 with broth was used at the base of each type of agar slant. Yeast extract plus autoclaved red-blood extract was also used. No growth occurred at the base of the nonnutrient agar slants in either the serum or the yeast medium, but growth did occur at the base of the nonnutrient salt agar slants (table 3).

[14] Unpublished data.

TABLE 3.—*Growth of the fowl coryza bacillus when 1.5 percent of nonnutrient agar and 1.5 percent of nonnutrient agar plus 0.8 percent of sodium chloride were substituted for nutrient agar*

Medium	24- and 48-hour microscopic examination [1]		Growth in check medium [2]	
	Culture 44	Culture 80	Culture 44	Culture 80
Yeast extract and autoclaved blood extract at base of nonnutrient agar slants	−	−	−	−
Yeast extract and autoclaved blood extract at base of nonnutrient salt agar slants	+	+	++	++
Sheep serum and broth (1:20) at base of nonnutrient agar slants	−	−	−	−
Goat serum and broth (1:20) at base of nonnutrient agar slants	−	−	−	−
Sheep serum and broth (1:20) at base of nonnutrient salt agar slants	+	+	++	++
Goat serum and broth (1:20) at base of nonnutrient salt agar slants	++	++	++	++

[1] +=growth; ++=good growth; −=no growth.
[2] Check medium consisted of chicken blood at base of nutrient agar slants.

TABLE 4.—*Effect on growth of the fowl coryza bacillus of the addition of 2 percent of sodium chloride to the growth medium*

Medium	24- and 48-hour microscopic examination [1]		Growth in check medium [2]	
	Culture 44	Culture 80	Culture 44	Culture 80
Horse serum and 2 percent NaCl broth (1:20)	−	−	−	−
Cow serum and 2 percent NaCl broth (1:20)	−	−	−	−
Sheep serum and 2 percent NaCl broth (1:20)	+	+	++	++
Rabbit serum and 2 percent NaCl broth (1:20)	−	−	−	−
Goat serum and 2 percent NaCl broth (1:20)	+	+	++	++
Pig serum and 2 percent NaCl broth (1:20)	−	−	−	−
Turkey serum and 2 percent NaCl broth (1:20)	+	+	++	++
Chicken serum and 2 percent NaCl broth (1:20)	++	++	++	++
Horse serum and 2 percent NaCl broth (1:5)	+	+	++	++
Cow serum and 2 percent NaCl broth (1:5)	−	−	+	+
Rabbit serum and 2 percent NaCl broth (1:5)	−	−	++	++
Pig serum and 2 percent NaCl broth (1:5)	−	−	−	−
Yeast extract, and 2 percent NaCl, and autoclaved red blood cells	−	−	−	−
Yeast extract, 2 percent NaCl, and autoclaved red blood cells at base of nutrient agar slants	++	++	++	++

[1] +=growth; ++=good growth; −=no growth.
[2] Check medium consisted of chicken blood at base of nutrient agar slants.

Table 4 shows the results of studies in which a 2-percent sodium chloride broth was used with the serum of the various animals in the proportion of 1:20 to determine whether the agar could be completely replaced by it. The 1:20 dilutions of the horse, cow, rabbit, and pig serums did not support growth, but the goat, sheep, turkey, and chicken serums did support it. Later, the serum from the horse, cow, rabbit, and pig were used with a 2-percent sodium chloride broth in the proportion of 1:5. No growth could be detected microscopically except in the horse serum, but the organisms were recovered from the cow and rabbit upon the inoculation of chicken blood at the base of nutrient agar slants. Yeast extract plus 2 percent sodium chloride and autoclaved blood extract failed to show growth, but when the same material was used at the base of nutrient agar slants growth occurred.

To determine the optimum proportion of sodium chloride for growth of the organism, chicken serum in the proportion of 1:20 in broth without salt, and in broth containing 0.5, 0.8, 1.5, 2.0, 2.5, 3.0 and 3.5 percent of salt was tested. Chicken serum diluted with broth in the proportion of 1:20 without salt was used at the base of nutrient agar slants to which no sodium chloride was added and with the nutrient agar containing the same percentage of salt as the broths mentioned above.

TABLE 5.—*Growth of the fowl coryza bacillus in chicken serum broth (1:20 dilution) and on nutrient agar when different percentages of salt were added*

Chicken serum [1] diluted (1:20) in broth plus—	Microscopical examination after [2]—			
	24 hours		48 hours	
	Culture 44	Culture 80	Culture 44	Culture 80
No NaCl	−	−	−	−
0.5 percent NaCl	+	+	+	+
Nutrient agar, no NaCl	−	−	−	−
Nutrient agar plus 0.5 percent NaCl	+	+	+	+
Broth plus 0.8 percent NaCl	+	+	+	+
Nutrient agar plus 0.8 percent NaCl	+	+	+	+
Broth plus 1.5 percent NaCl	+	+	++	++
Nutrient agar plus 1.5 percent NaCl	+	+	++	++
Broth plus 2 percent NaCl	++	++	++	++
Nutrient agar plus 2 percent NaCl	++	++	++	++
Broth plus 2.5 percent NaCl	±	±	±	±
Nutrient agar plus 2.5 percent NaCl	±	±	±	±
Broth plus 3 percent NaCl	×	×	×	×
Nutrient agar plus 3 percent NaCl	±	±	±	±
Broth plus 3.5 percent NaCl	×	×	×	×
Nutrient agar plus 3.5 percent NaCl	×	×	×	×
Control broths, no serums [3]	−	−	−	−

[1] Chicken serum diluted 1:20 with broth without salt was used at the base of the nutrient agar slants.
[2] +=growth; ++=good growth; −=no growth; ±=poor growth; ×=growth obtained in chicken blood at the base of nutrient agar slants after being inoculated from these test media.
[3] Controls consisted of broth with and without the respective amounts of salt used in each instance.

The results (table 5) indicate that salt is essential for growth. The growth ranges from the 0.5 percent salt in the broth and agar up to 2.5 percent for the broth and to 3.0 percent for the agar, the optimum concentration ranging between 1.5 and 2.0 percent for both the broth and agar.

These tests indicate that sodium chloride contributes to the growth of the organisms as employed, but does not completely take the place of agar. That nutrients of the nutrient agar are not necessarily essential is also evident from these trials. Whether other salts could replace the sodium chloride has not been determined.

SUMMARY AND CONCLUSIONS

The need of the X factor for the growth of the Rhode Island strains of the fowl coryza bacillus is indicated by the failure of the organism to grow in yeast extract or yeast suspensions at the base of nutrient agar slants unless this factor is supplied in some manner, as by the addition of autoclaved blood extract, or when the medium used to inoculate the yeast extract is of blood and sufficient of the X factor is carried over in the inoculum.

Growth on yeast extract prepared by boiling was poorer than that on yeast extract prepared without heat. Yeast-cell suspensions plus autoclaved blood extract supported growth. Yeast-cell suspensions alone did not.

The clotted-blood serum of the horse, cow, sheep, goat, pig, and rabbit failed to support growth when employed in dilutions of 1:20 in broth, but the same serum broth supported growth when used at the base of nutrient agar slants. This indicates that the growth factors were not so concentrated in the serum of these animals as in those of the chicken and turkey, but that this was partly compensated for when nutrient agar was employed; although, as shown by the controls, neither the agar nor the broth was capable of supplying these requirements. The serum of the horse, cow, rabbit, and pig in a dilution of 1:20 at the base of agar slants failed to give continued growth when transfers were made to additional tubes of the same serum medium, whereas continued cultivation was possible in the case of the sheep, goat, turkey, and chicken serums.

A study of the role of agar in growth indicated that sodium chloride in the proportions used could partly but not completely replace agar in furnishing the requirements necessary for growth.

That the factors in chicken serum in a dilution of 1:20 are adversely affected by boiling but are not completely destroyed is indicated by the fact that some growth occurred when the serum was used at the base of nutrient agar slants.

The results of the tests with the serum of horse, sheep, goat, cow, and rabbit, when used in the dilution of 1:20 in broth, were similar to those reported by Kessens.[15] That the same serum broths at the base of agar slants supported growth in some degree indicates that they were not entirely free from the growth factors.

The results of the use of unheated yeast extract indicate that *Hemophilus gallinarum* will not grow when only the V and C factors are present. The need of the X factor is indicated by the results obtained when autoclaved blood extract was added to the yeast extract. Boiled yeast extract plus autoclaved blood extract does not support good growth although it contains the X and V factors.

Considerable differences in growth were noted in yeast extracts and serum broths when used alone and when used at the base of nutrient agar slants.

Sodium chloride concentrations up to 2 percent was important in the growth of the organism and partly accounted for the results obtained in using the nutrient agar slants.

No appreciable difference in growth was noted between the two strains of the organism that were used, although one had only recently been isolated and the other had been maintained in culture for more than 3 years.

[15] KESSENS, B. H. See footnote 2.

A CRITICAL EVALUATION OF THE RAT-GROWTH METHOD FOR DETERMINING VITAMIN B AND ITS CONTENT IN MEALS FROM CERTAIN OILY SEEDS [1]

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INTRODUCTION

There is little published information on the amount of vitamin B in the more common oil press-cake meals, namely, cottonseed meal, linseed meal, soybean meal, and peanut meal. Morrison (*14, p. 1013*)[2] rates the amount present in peanut meal and in soybean meal as++. Eddy and Dalldorf (*6*) state that cottonseed meal contains 65 International units of vitamin B per ounce. Others (*18, 21*) have reported that cottonseed meal is a good source of this vitamin, but they do not give quantitative figures. Munsell and DeVaney (*15*) found that cottonseed flour contains 5 Sherman units of vitamin B per gram.

The assays reported here were made between 1929 and 1936 by the rat-growth method. In the early work no control rats on a standard of reference were used. Later a sample of dried brewers' yeast was adopted as a standard and fed in parallel with the meals. When the international vitamin B standard became available in 1932 this reference yeast was assayed in terms of International units of vitamin B.

Since the earlier assays could not be evaluated by direct comparison with a standard, it was of interest to determine whether some relation between growth response and vitamin B dosage could be found by which reasonably accurate estimates of the vitamin B potency could be made.

EXPERIMENTAL PROCEDURE

Rats, 22 to 26 days old and weighing 40 to 50 g., were fed vitamin B-free basal ration until they ceased to gain in weight, usually 20 days. They were then placed in individual cages, having raised screen floors, and fed the basal ration and distilled water, ad libitum, together with a definite daily dosage of either the meal being tested or the reference standard, for 7 weeks. The rats were weighed thrice weekly.

The basal ration of Hunt and Krauss (*10*) as modified by Sherwood and Halverson (*17*) was used. It consisted of purified casein (*9, p.122*), 18 percent; washed corn starch, 64 percent; hydrogenated vegetable fat, 10 percent; agar-agar, 2 per cent; cod-liver oil, 2 percent; and McCollum's salt mixture No. 184, 4 percent. The salt mixture was later changed to that proposed by Wesson (*20*), 3.5 percent, and the starch was increased to 64.5 percent. In addition each rat received daily 0.3 g. of autoclaved dried brewers' yeast which had been found to be free from vitamin B.

[1] Received for publication October 11, 1937; issued June, 1938. Read before the Symposium on the Vitamin B Complex, Ninety-third Meeting of the American Chemical Society, Chapel Hill, N. C., April 13, 1937.

[2] Reference is made by number (italic) to Literature Cited, p. 933.

ESTIMATING EQUATIONS

There is no unanimity of opinion as to the form of an equation for expressing the relation between gain response and vitamin B dosage. Supplee, Bender, and Flanigan (*19, p. 373*) state that there is "a direct linear relationship between the rate of growth of white rats and the amount of vitamin B supplied in the ration." Coward, Burn, Ling, and Morgan (*2*) concluded that there is a linear relation between the logarithm of the vitamin B dosage per day and the total gain in the feeding period. Schlutz and Knott (*16*) found that their results indicated a linear equation relating the logarithm of the total vitamin B eaten to the logarithm of the total gain in a given period. Dann and Cowgill (*5*) state that the vitamin B requirement for growth of female rats is proportional to the five-thirds power of the weight.

Preliminary calculations indicated that linear equations of the type

1: International units of vitamin B per day $= a + b$ (total gain) and

2: Logarithm (International units of vitamin B per day) $= a + b$ (total gain)

seemed to fit the data for the rats getting the International standard better than others tried. Equation 1 is similar to that proposed by Supplee, Bender, and Flanigan, and 2 is Coward's equation. The type proposed by Schlutz and Knott did not fit the results very well, the coefficient of correlation for a 4-week feeding period being 0.49.

Table 1 shows the least-square solution of equations of types 1 and 2 for a 4-week feeding period. It also gives the coefficients of correlation and the standard errors for feeding periods of 2 through 7 weeks as computed from the results with the rats receiving the International vitamin B standard.

The results at the end of the first week are not given in table 1 because computation of the coefficients of variation in gain at weekly intervals for different groups of rats at different levels of the International vitamin B standard or of either of four samples of dried brewers' yeast, invariably showed that the variation in gain was greater at the end of the first week than at any subsequent period. After the second (occasionally the third) week there was little change in the coefficient of variation provided the vitamin B dosage was above the maintenance level.

TABLE 1.—*Least-square solution of equations of types 1 and 2 for a 4-week feeding period, and coefficients of correlation and standard errors for feeding periods of from 2 to 7 weeks computed from results with rats receiving the International vitamin B standard*

Assay period (weeks)	Rats	Correlation coefficients		Standard error	
		Arithmetic	Semilog-arithmic	Arithmetic	Semilog-arithmic
	Number				
2	49	0.75	0.77	0.22	0.112
3	49	.71	.77	.23	.112
4	46	.79	.92	.20	.062
5	44	.80	.82	.20	.101
6	40	.86	.85	.17	.091
7	37	.83	.79	.13	.102

Estimating equations—4 weeks:
1, arithmetic; International units of vitamin B=0.0148 (gain)+0.606.
2, semilogarithmic; log. International units of vitamin B=0.0080 (gain)−0.2367.

The coefficient of correlation based upon the arithmetic relation between gain and dosage apparently increases with the length of the test period. However, calculation of the significance of the differences between these coefficients by means of Fisher's z transformation (*8, p. 182*) shows that they are not significant but may be due to chance. The coefficients based on the semilogarithmic relation (equation 2) seem to reach a maximum at 4 weeks, but again there is no *significant* difference between them. Both sets of correlation coefficients have about the same order of magnitude. When tested by the analysis of variance method (*8, p. 232*) neither of the equations in table 1 is found to differ significantly from the linear. For these reasons there is little choice between these two types of estimating equations.

LENGTH OF FEEDING PERIOD

Because of the seasonal variation in response (discussed later), the comparatively small number of rats used, and the absence of significant differences between the correlation coefficients for the different time intervals, this analysis does not show definitely the optimum length of test period. It indicates that a 2-week period is about as accurate as a longer one. Coward (*1*) has concluded from her studies on a much larger number of rats that a 2-week feeding period yields more accurate results than a 1-week test and that "the increased accuracy obtained by carrying on a test for longer than two weeks is seldom worth the extra labour involved." Schlutz and Knott (*16*) have found that a 10-day feeding period yields valid results provided certain precautions, which they enumerate, are observed. On the other hand, Lassen (*12*) concludes that a 5-week feeding period yields the smallest mean error.

More or less arbitrarily the arithmetic equation for the 4-week period (equation 1, table 1) was used in estimating the vitamin B content of those meals that were tested before the known standards of reference were adopted. Assuming that environmental and other uncontrolled factors did not vary enough to invalidate the application of this equation to the earlier results, the standard error (0.21) shows that the vitamin B potency calculated from the growth response of a single rat will not vary from the true value by more than ± 0.42 of an International B unit in 95 percent of the trials. In the later assays 10 rats were used on each of at least 2 dosage levels of the meals; but in the earlier work there were usually only 6 replicates. The accuracy of the equation with a probability of 0.05, for six rats, is twice the standard error divided by the square root of $6-1$, or approximately ± 0.2 of an International unit of vitamin B.

VITAMIN B IN OIL PRESS-CAKE MEALS

The results of the vitamin B assays are given in table 2. The sample of cottonseed flour, one sample of cottonseed meal, one of soybean meal, and two of peanut meal were run without reference standards, hence the vitamin B content was computed by means of the estimating equation. The potency of the raw, shelled, Virginia Runner peanuts was calculated from previously published (*17*) growth-response data from this laboratory. Daniel and Munsell (*4*)

TABLE 2.—*International units of vitamin B per gram in press-cake meals and other products*

Product	Samples	International units of vitamin B per gram	
		Mean	Range
	Number		
Soybean meal	4	1.5	1.1–1.7
Soybean meal, solvent process	1	3.9	----------
Meal from Spanish peanuts	2	2.7	2.6–2.7
Meal from Virginia Runner peanuts	2	2.5	2.4–2.6
Cottonseed meal, 36 percent protein	5	3.8	3.0–5.2
Cottonseed meal, 41 percent protein	1	5.0	----------
Cottonseed flour (Allison flour)	1	4.4	----------
Linseed meal	3	4.3	3.3–5.4
Raw Virginia Runner peanuts	1	2.4	----------
Cottonseed-hull bran	1	0	----------

have used these same data for computing the vitamin B content of peanuts in terms of Sherman units. The amount of vitamin B in cottonseed-hull bran was too small to be detected when fed at a level of 1 g per day.

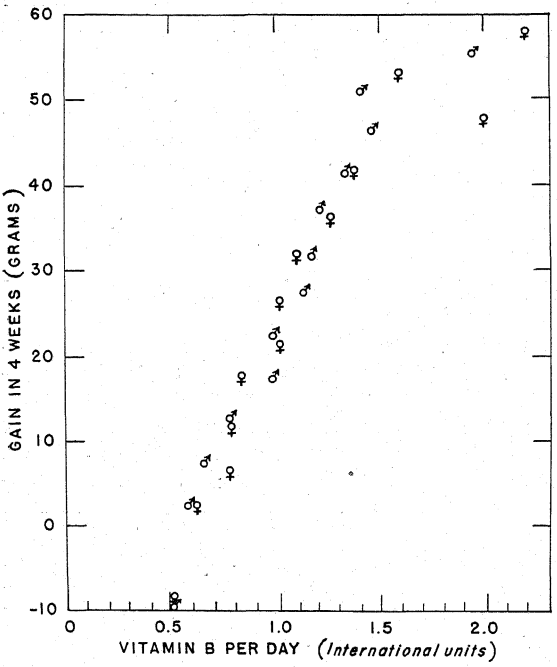


FIGURE 1.—Relation between vitamin B dosage and gain response according to the sex of the test rat.

RELATION OF SEX TO GROWTH RESPONSE

During the course of these assays an impression was obtained that the sex of the rat had little effect on the gain, although approximately equal numbers of males and females were used in each test. In order to clarify this point the average daily vitamin B intake of each rat used in these assays (a total of 670 rats) was computed. These figures were classified according to the sex of the rat and then grouped according to the gain in 4 weeks. The mean gain and the mean vitamin B intake for each group were then determined and plotted, as shown in figure 1.

Figure 1 shows that there is no essential difference in the growth response of the two sexes.

Figure 1 illustrates also the linear relation between total gain and International units of vitamin B per day. The points fall approximately on a straight line except at the ends. The lower end shows

that 0.6 International unit per day is the minimum average amount required for maintenance and that losses in weight are not proportional to the vitamin B intake. Since comparatively few rats received as much as 1.6 International units per day no high degree of confidence can be placed in the extreme upper end of the curve, but the results indicate that the linear relation does not hold for females making gains of 45 g or more in 28 days or for males whose gains are more than 50 g. Apparently the growth response is slightly less for a given increase in vitamin B dosage above 1.5 units than it is when the dosage lies between 0.6 and 1.5 units per day. The fact that Coward's data (*1*) extended considerably beyond these limits may account for her finding a semilogarithmic relation between gain and vitamin B intake.

SEASONAL VARIATION IN GROWTH RESPONSE

General observation, while making the assays, indicated that there is a distinct seasonal variation in response to a given dosage of vitamin B. This variation is relatively unimportant when adequate controls on a known standard are used in parallel with the test animals. However, it does have a bearing on the interpretation of results when the vitamin B unit employed is based upon the growth rate or when a curve of reference is used to evaluate the results, as in the earlier assays reported here.

To determine seasonal variation, a joint-function analysis between the vitamin B dosage of all 670 rats used in the assays and the gain in 28 days and the month in which the assay was started was made by the method described by Ezekiel (*7, pp. 277–294*). The results are summarized in figure 2.

This series of curves shows a distinct seasonal variation in growth response for a given daily dose of vitamin B. From September to February 0.6 of an International unit of vitamin B per day is not sufficient to supply the maintenance requirements of the rats. Beginning in February and continuing to June, this amount of the vitamin promotes increasing gains, but about the first of July the needs of the animals increase so that by the first of September 0.6 of a unit is just sufficient for maintenance of weight. In June 0.6 of a unit promotes a gain of approximately 3 g per week, but in October 0.85 of a unit is required for the same gain. With larger doses of vitamin B the effect of seasonal variation becomes less marked and the peaks and valleys of the curves are shifted somewhat to the left.

Since the data for the individual years varied somewhat from the averages shown, the curves in figure 2 should not be used as curves of estimate. Their use may yield fair approximations of vitamin B potency, but neither these curves nor the estimating equations given above are adequate substitutes for controls on a reference standard, maintained as a part of the assay.

While the seasonal variation in vitamin B requirement does not coincide with the variation in the gains of normal rats as found by Levene (*13*), it is interesting to note that Cowgill (*3, p. 136*) quotes Braddon as saying relative to beriberi in the Singapore Criminal Prison, "* * * an outbreak occurred, as in former years, in August." Also, it is reported (*11, p. 22*) that a condition apparently due to a deficiency of vitamin B becomes common among infants in Palestine with the approach of hot dry winds.

SUMMARY

Soybean meal, peanut meal, cottonseed flour, cottonseed meal, and linseed meal have been found to be good sources of vitamin B. The values ranged from 1.1 International units of vitamin B per gram for a sample of soybean meal to 5.4 International units per gram in one sample of linseed meal. The other meals had intermediate values.

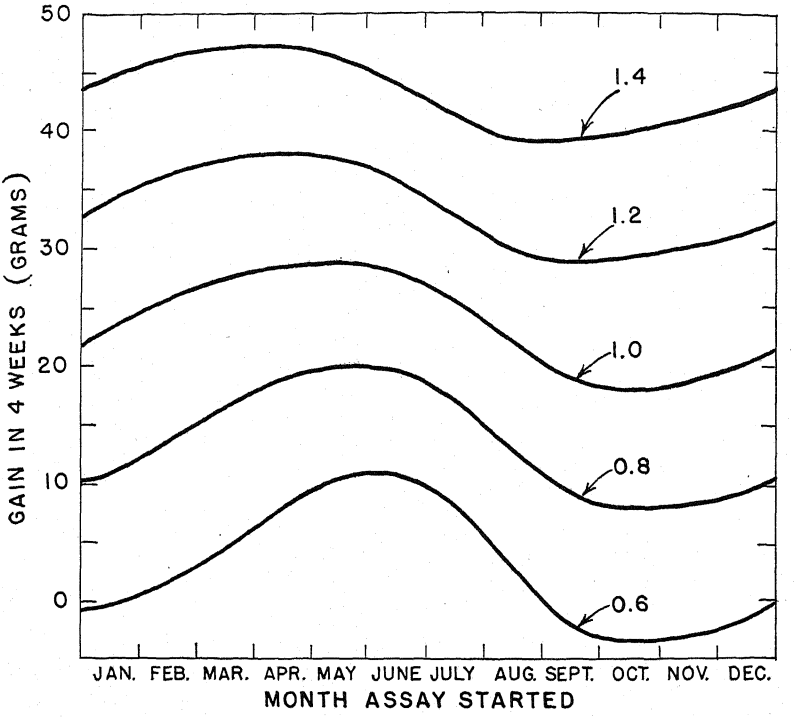


FIGURE 2.—Seasonal variations in gain of rats on different levels of vitamin B. Figures indicate dosage in International units.

Cottonseed-hull bran did not contain an appreciable amount of vitamin B.

Raw shelled peanuts were found to contain 2.4 International units per gram when old data were interpreted by means of the estimating equation given in the text.

A statistical study of the results of the assays by the rat-growth method shows that:

(1) A 7-day feeding period is not long enough for the most accurate results.

(2) There is no advantage in continuing the assay longer than 3 weeks.

(3) The relation between vitamin B dosage per day and total gain in the assay period may be expressed by a linear equation, provided the total gain is less than 50 g in 4 weeks and the daily vitamin B dosage lies between 0.6 and 1.4 International units.

(4) When the vitamin B dosage is within the limits just given there is no difference in response due to the sex of the rat.

(5) There is a seasonal variation in growth response to a given dose of vitamin B. This variation is most marked when the amount of vitamin B is only slightly greater than that required for maintenance. Rats apparently need less vitamin B in the spring and early summer than in late summer and autumn.

(6) A curve of reference may be helpful in interpreting the results of an assay, but it should not be substituted for adequate controls on a reference standard.

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